



1977

Observation of Long and Short Term Effects of Capsicum (Red Pepper) and Capsaicine on the Morphology and the Biochemistry of the Albino Rat Stomach

Gabriel John Ekandem
Loyola University Chicago

Recommended Citation

Ekandem, Gabriel John, "Observation of Long and Short Term Effects of Capsicum (Red Pepper) and Capsaicine on the Morphology and the Biochemistry of the Albino Rat Stomach" (1977). *Dissertations*. Paper 1667.
http://ecommons.luc.edu/luc_diss/1667

This Dissertation is brought to you for free and open access by the Theses and Dissertations at Loyola eCommons. It has been accepted for inclusion in Dissertations by an authorized administrator of Loyola eCommons. For more information, please contact ecommons@luc.edu.



This work is licensed under a [Creative Commons Attribution-Noncommercial-No Derivative Works 3.0 License](https://creativecommons.org/licenses/by-nc-nd/3.0/).
Copyright © 1977 Gabriel John Ekandem

210

OBSERVATION OF LONG AND SHORT TERM EFFECTS OF CAPSICUM (RED PEPPER)
AND CAPSAICINE ON THE MORPHOLOGY AND THE
BIOCHEMISTRY OF THE ALBINO RAT STOMACH

By

Gabriel John Ekandem

A Dissertation Submitted to the Faculty of the Graduate School
of Loyola University of Chicago in Partial Fulfillment
of the Requirements for the Degree of
Doctor of Philosophy

June, 1977

BIOGRAPHY

Gabriel John Ekandem was born in Nigeria, on September 29, 1943. He graduated from St. John Fisher College, Rochester, New York, with a B.S. in Biology. He received his M.S. degree in Biology from Loyola University of Chicago, in 1974.

He began his studies for Ph.D. in the Anatomy Department, Loyola University of Chicago, Stritch School of Medicine, Maywood, Illinois in July 1973.

Gabriel is planning to return to his country (Nigeria) soon after completing his Doctoral degree.

ACKNOWLEDGEMENTS

It is my pleasure to express my deep and sincere gratitude to a number of individuals who have been instrumental in the successful completion of my graduate studies. First, I wish to thank Dr. Leslie A. Emmert, my advisor for his suggestions and counsel during the undertaking of the research and dissertation. I wish also to thank the other members of the dissertation committee, Dr. Martin Durkin, Dr. James McDonald, Dr. Charles O'Morchoe, Dr. Harold Manner and Dr. Sigfrid Zitzlsperger for their counsel and careful consideration of the dissertation. I wish to thank Mr. Hiroshi Tonaki of the Department of Electron Microscopy, V.A. Hospital, Hines, Illinois for advice and counsel on scanning and transmission electron microscopy. I wish to thank Rev. Fr. Matthew Creighton, Associate Dean, and Rev. Fr. Raymond Hayes, Vice President, for making it possible for me to pursue my graduate studies at Loyola University.

Finally, I wish to thank those of my family who have sacrificed much to make possible the continuation and successful completion of my studies. Special thanks to my wife, Alice, for the great patience and unending help whenever she could provide it, including moral support and winning bread for the family throughout my years of study. My son, Utibe, who was born here in the U.S.A., has been a great inspiration by his very presence.

TABLE OF CONTENTS

	Page
TITLE OF DISSERTATION.....	I
BIOGRAPHY.....	II
ACKNOWLEDGEMENT.....	III
TABLE OF CONTENTS.....	IV
ABSTRACT.....	V-VII
INTRODUCTION.....	1-6
REVIEW OF THE LITERATURE.....	7-12
MATERIALS AND METHODS.....	13-21
EXPERIMENTAL RESULTS.....	22-34
DISCUSSION.....	35-40
SUMMARY.....	41-43
LITERATURE CITED.....	44-58
FIGURES AND LEDGENDS.....	59-188
COMMITTEE ON CANDIDACY/APPROVAL SHEET.....	189

ABSTRACT

The long term effects of *Capsicum* (red pepper) and its active principle, capsaicine, on the morphology of the gastric mucosa were studied in adult albino rats which were fed daily by intubation either an aqueous extract of *Capsicum annum* or a solution of capsaicine in 5% ethyl alcohol for periods of three, six or nine months. Control rats received similar volumes of either water or 5% ethyl alcohol. At the end of the respective time period, the stomachs were removed and examined both grossly and by light microscopy.

In order to study the short term effects of capsaicine on the mucosa of the empty stomach, a closed gastric pouch was created under nembutal anesthesia in adult rats by ligating the pyloric and esophageal openings and incising the stomach along the greater curvature to permit flushing out of the gastric contents and the introduction or withdrawal of solution. Immediately upon introduction of a solution a sample was withdrawn to serve as a control and the exposure was continued for periods of 30, 60 or 90 minutes, at which times final samples were taken for chemical analysis for K^+ , Na^+ , Cl^- , pH and Hemoglobin. The stomachs were processed for study by light and electron microscopy, both scanning and transmission.

Exposure to water or 5% alcohol alone failed to elicit the changes observed upon exposure to the capsaicine solution. The amount of capsaicine utilized in both long and short term studies, one

milligram per kilogram of body weight, approximates the amount consumed in a single typical meal in Thailand. In the rat, this concentration has proven to be an irritating cytotoxic substance as shown by the following changes observed in both long and short term studies: (1) The loss of the mucous film covering the free surface of the lining epithelium, (2) the degeneration and sloughing of the mucous secreting cells covering the luminal surface and lining the gastric pits, thus denuding the connective tissue and capillaries of the tunica propria, (3) edema of the mucosa and submucosa, (4) hyperemia leading to capillary distention and disruption with the extravasation of blood into the tunica propria connective tissue space and culminating in frank bleeding into the gastric lumen. Initially, these alterations are small and focal in distribution becoming larger and more numerous with longer exposure. The most wide spread involvement was observed in animals having been fed capsaicine for nine months.

Analysis of capsaicine exposed gastric pouch contents showed significant increases in Na^+ , K^+ , Cl^- and hemoglobin, but a decline in H^+ when compared with control values. Significant differences in the same direction were observed when different time groups were compared. Utilizing the hemoglobin concentration as an index of the amount of bleeding into the pouch together with standard rat blood values for electrolytes, a calculation has shown that the role of blood in the pouch is not the only one in eliciting these changes.

It is concluded that capsaicine, in addition to producing morphological alterations, has altered the physiological properties of the gastric mucosa.

INTRODUCTION

The geographical distribution of a disease may provide valuable clues with regard to its etiology. Likewise, any historical changes in prevalence, associated with changes in the mode of living, may give additional information. The prevalence of duodenal ulcer in Africa, south of the Sahara, has been a subject of much speculation and although the medical personnel, mostly foreign, who work in this area, have always tried to provide a cure, very little has been done to find the cause.

The World Health Organization (WHO) collects the information shown in Figure 1 (Lagos Health Report, Nigeria, 1972), about duodenal ulcers in Africa in several ways: by reviewing all the available literature; by extensive correspondence, personal interviews and visits; and by replies to questionnaires sent out by WHO to a large number of mission hospitals, many of which have sent monthly returns over a period of years.

There are many difficult problems to overcome in trying to establish the prevalence of a disease with a low mortality such as duodenal ulcer. These problems are considerable in a developed country and much greater in developing countries. The medical personnel have endeavoured only to establish whether duodenal ulcer is a common or a rare problem in a given area. It has not been possible for the most part to use any exact parameters. In making an assessment it was noted whether the diagnosis had been made on clinical findings,

x-ray evidence, surgical experience, or necropsy examination (Lagos Health Report, 1972). Many hospitals are without x-ray facilities. Surgical statistics can be selective and misleading, depending often on the facilities available and the reputation of the hospital, but nonetheless can be a valuable guide. One of the most useful indicators has been the incidence of complications - pyloric stenosis, hemorrhage and perforation, none of which can easily be overlooked. Great value has been attached to reports from mission hospitals where there has been long continued service by individual doctors and where records have been well kept. Wherever possible the number of proven duodenal ulcer cases has been related to the number of annual admissions (excluding maternity). Figure 1 presents the overall results of the survey. Areas in which duodenal ulcer is common, occurs occasionally, or is uncommon, are indicated.

The existence of high and low prevalence areas is confirmed. High prevalence areas occur along the west coast, in the Nile-Congo watershed, in northern Tanzania and in Ethiopia. Along the west coast, the highest prevalence areas is in the eastern area in the Cameroons, Nigeria and on into Ghana. There is some evidence that it is higher in the rain forest than in the coastal zone. The prevalence is much lower to the north in the savannah regions. There are peculiar pockets of low incidence in the coastal zone, eg, in eastern Nigeria, home of the author, and in Ghana, around Adidome,

which are hard to explain. The latter area, however, is dry and described as "costal savannah" (Lagos Health Report, 1972).

The highest prevalence of all seems to be in the Nile-Congo watershed, where duodenal ulcer surgery forms the major part of all abdominal surgery. The area includes Rwanda and Burundi, eastern Zaire around Lake Kivu, extreme western Tanzania adjacent to Burundi, and the south-western Uganda. Thus, at Matania in Burundi, a 58 bedded, one doctor hospital, 780 operations for peptic ulcer were done in 10 years (79% of all major surgery), and at Buye, a 76 bedded hospital, 404 operations for peptic ulcer were done in a period of two years eight months. Duodenal ulcer also occurs frequently in the Wachaggas around Kilimanjaro in northern Tanzania. In Ethiopia the incidence is high in the highlands extending from Addis Ababa up to Condar and Asmara. It is rare in the lower country to the south where maize, millet and wheat are grown (Lagos Health Report, 1972).

As stated above, medical personnel who work in these parts of Africa have endeavored only to establish whether duodenal ulcer is a common or rare problem. The cause remains a mystery. It was the search for the cause of duodenal ulcer which is so prevalent in the home town of this author that stimulated his work on red hot pepper. Red pepper is used on every food eaten in this part of the world. The ingestion of large quantities of red pepper may contribute to the high incidence of gastrointestinal (g.i.) ulcers in Nigeria and in

other African countries south of the Sahara.

The present study was undertaken to investigate in rats the histopathologic and biochemical changes of the stomach induced by long term administration of large doses of red pepper and its active substance, capsaicine. The study also investigated the direct effect of capsaicine on a 30 minute, 60 minute and 90 minute period. A description of events occurring in the injury is presented. Injury increased with the duration of exposure.

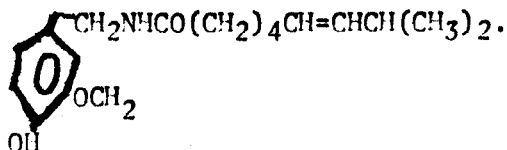
Capsaicine is a pungent principle present in various species of *Capsicum* (hot pepper). The fruit of several species of *Capsicum* e.g., *Capsicum frutescens*, *Capsicum minimum*, *Capsicum grossum* and *Capsicum annum*, play an important part in the dietary of most African people. The people get so used to the sharp taste of red hot pepper that they virtually lose their appetite in its absence; and although several ailments are attributed to excessive use of the spicy fruit, such as bloody stool, vomiting, "internal heat", running nose and tears, most people (including the author of this investigation) would rather run the risk than do away with the wanted pungency.

Hot green or red pepper is the most common spice used in food throughout the world, especially in Africa, Latin America and Asia. Isolation of capsaicine from red pepper was successfully carried out by Toh et al in 1954 and by Kosuge et al in 1958. Hot red pepper contains approximately 0.1 - 1.0% capsaicine (Sirsat and Khanolkar, 1960), a pungent and pain-producing chemical substance. Capsaicine is freely soluble in hot water (Nelson and Dawson, 1923), and slightly soluble in cold.

In Nigeria, it serves as a universally used spice and forms the principal ingredient of many sauces and condiments. Red hot pepper graces the table of the richest as well as that of the poorest. Commercially hot pepper is sold under different names: cayenne pepper, African chillies, tabasco pepper, curries, paprika, Louisiana long pepper or Louisiana sport pepper which is a hybrid between the Honka variety of Japanese Capsicum and the old Louisiana sport pepper. In the continental U.S.A., National Formulary (N.F.) requires Capsicum to be labelled to indicate which of the above varieties is contained in the package. Its active principle, capsaicine, is quite stable, persisting in apparently unreduced potency in dried peppers. There is a general feeling among physicians in Asia (Lee So. 1963) that the pungent principle, capsaicine, of this hot pepper causes gastrointestinal disorders..

In Germany and in Bulgaria, capsaicine is used as insecticides. Also in Bulgaria, capsaicine is used to activate tumor-inducing ability of certain forms of bacteria in plants.

Hot red peppers contain capsaicine, a pungent and pain producing chemical substance. Capsaicine is the vanillylamide of 8-methyl-6 nonemic acid with the formula: $C_{18}H_{27}NO_3$: Mol. wt. 305.40



For many centuries man has used red pepper, but it is just recently that capsaicine has captured the attention of a few investigators

because its biting, burning properties suggest it could have pharmacological and physiological effects. However, direct effects of capsaicine on the gastrointestinal tract has received less attention than the pharmacological and physiological effects.

It is the aim of this author to demonstrate that Capsicum solution upon direct contact with gastric mucosa can produce pathological changes ranging from edema, hyperemia, congestion, submucosal hemorrhage to overt or frank bleeding.

In this investigation, Capsicum annum, which is noted for its extreme pungency, and capsaicine are used. The effects of these substances on the morphology and biochemistry of rats stomach are reported.

REVIEW OF THE LITERATURE

Inorganic, as well as organic substances, including some therapeutic agents, have been reported to produce damage to the gastric mucosa.

Toerell (1939) observed that acetic acid "inflames" the mucosa, and there is a large literature on the effects of acetylsalicylic acid (ASA) on the stomach (Levy and Hayes, 1960; Rote et al 1963). Acetic, propionic and butyric acids have been shown to damage the gastric mucosa (Davenport, 1964). The scanning electron microscopic (SEM) study of Frenning and Obrink (1971) on the effects of ASA on cat gastric mucosa also described severe apical damage. Studies of the effects of cytotoxic agents such as ASA (Hingson and Ito, 1971), bile salts (Eastwood, 1974) and ethanol (Eastwood, 1975) on the integrity of the gastric surface epithelium showed severe cytoplasmic degradation. Harding and Morris (1976) have studied pathological effects of aspirin and hemorrhagic shock on the gastric mucosa of the rat. Topographical examination of gastric fundic epithelia revealed that significant morphological differences occur not only between species and between individuals exposed to the same experimental conditions, but also between identically treated samples taken from the same animal. Exposure to aspirin produced apical erosions, bleeding and loss of granules. Hemorrhagic shock produced erosions and degeneration of superficial epithelial cells.

Some other substances evaluated included neomycin (Jacobson, 1950), fluoride and mersalyl (Bond and Hunt, 1956), methothrexate (Trier, 1962), aminopterin (Millington, 1962 and Ryback, 1962), 0.2 N HCl, 0.5% formic

acid, and glucose (Williams, 1963), puromycin (Estensen and Baserga, 1966) and cyclohexamide (Allen, 1971). Nitrogen mustard and radiation have also been reported to cause some damage to the gastric mucosa cells (Rubin, 1971).

Capsicum which is ground up red hot pepper, and capsaicine, the active principle extracted from red hot pepper, have been shown to produce damage to the mucosa of some parts of the G.I. tract and to some parts of the nervous system. Submucosal fibrosis of the palate is found in many parts of India. Sirsat and Khanolkar (1960) using Wister rats attempted to induce submucosal fibrosis to the palate by local application of capsaicine. Immediately on painting the palate, the animals showed temporary signs of distress. The epithelium of the palate of buccal mucosa of the animals showed hyperplasia and hyperkeratosis. The subepithelial layers showed degeneration of connective tissue. From this work it was concluded that the pathogenesis of submucosal fibrosis of the palate might possibly lie in the continuous action of some irritant (possibly capsaicine) over a prolonged period of time.

The influence of Capsicum solution on gastric acidities was reported by Ketushinh et al (1966). Four human volunteers served as the subjects, and all were moderate users of Capsicum. The subjects fasted for 24 hours and the residual stomach contents emptied by means of a simplified Ryle tube. The subjects swallowed Capsicum solution after which gastric juice was withdrawn at regular intervals until eight samples more had been collected. The samples were filtered and titrated against standardized N/10 NaOH. Distilled water was used as

the control. They reported that after administration of Capsicum the free acidity rose to and persisted at a noticeably higher level than with water. All subjects reported the Capsicum solution to be pungent, but not excessive while in the mouth. In the stomach, however, there was a sensation of local warmth followed by slight burning pain. A few minutes later the warm feeling spread to upper limbs and face.

Joo et al in 1969 administered capsaicine solution subcutaneously in the rat. Mitochondrial alterations were observed in Type A spinal ganglion cells. They speculated that a functioning disturbance must occur in these cells following mitochondrial alterations induced by capsaicine.

Viranuvatti et al in 1972 made a gastroscopic observation of the effects of Capsicum on human gastric mucosa. Their aim was to demonstrate the direct local effects of Capsicum (red hot pepper) solution on the surface gastric mucosa by direct visual observation through the gastroscope. Twenty human subjects were selected for this study comprising of fifteen males and five females with ages ranging from 18 to 77 years. Any symptoms of abdominal discomfort or burning pain sensation were noted. The results of this work showed that when Capsicum solution was introduced into the stomach, the mucous surface became edematous, congested and minute submucosal hemorrhages occurred within a few minutes. They performed only gastroscopic observation lasting a few minutes; no chronic and no histological observations were made. They did no analysis of the gastric secretions.

Nopanitaya (1974) studied the effects of capsaicine and its combination with various diets on the morphology of the duodenal mucosa

of young rats. Normal diets with and without capsaicine were used. Goblet cells in the capsaicine-treated animals increased in number. Ultrastructural alterations occurred in absorptive cells of rats fed with diets supplemented with capsaicine.

Light and electron microscopic study of duodenal mucosal response to the active principle of hot red pepper in the rat was studied by Nopanitaya and Nye (1974). Both natural and synthetic capsaicine administered by the same route for the same length of time produced identical morphologic alterations. Injury increased with duration of exposure, and within two minutes, mitochondria were swollen. Increased numbers of free ribosomes and lysosomes and dilatation of endoplasmic reticulum and golgi complexes were evident. Nuclei were shrunk and the chromatin became marginated at nuclear envelope. No observations have been made on a direct damaging effect of capsaicine and Capsicum on the gastric mucosa of the rat. In Korea, Lee (1963) has studied the effects of red hot pepper on various organs of the rabbit, including the stomach. Unfortunately, this report is not available in U.S.A.

Physiological and pharmacological effects of Capsicum and capsaicine and related substances have been observed on various structures. Huebner (1925) found that undecylencidic allinylamide, an acrid substance related to capsaicine, caused sweating of the forehead of man when smeared upon the mucous membrane of the mouth.

In a study of gustatory sweating in healthy people, Lee (1954) used the fresh fruits of Capsicum frutescens. He produced evidence that the reflex was initiated by pain fibers and showed that reflex sweating could not be induced by application of Capsicum to the mucosa

of the esophagus or stomach. He found, however, that Capsicum produced profuse salivation when placed in the mouth.

Cardiovascular effects of capsaicine in dogs and rabbits were investigated by Toda et al (1971). In anesthetised dogs, intravenous injections of capsaicine caused a transient rise in mean systemic blood pressure followed by a sustained fall. In rabbits, capsaicine caused only a period of hypertension. They also observed that capsaicine caused constriction in isolated peripheral arteries.

Webb-Peploe (1972) studied the vascular response to stimulation of receptors in muscle by capsaicine. Dogs were used for this investigation. Capsaicine was injected into the iliac artery of dogs whose carotid sinus and vagus nerves were sectioned. There was an increase in perfusion pressure and increase in aortic and splenic pressures. These studies indicate that Capsicum and capsaicine are powerful substances which exert various influences.

The biochemical response of the stomach of some irritants has been investigated. On the permeability of the stomach mucosa for acids and some other substances, Teorell (1935) proved that the acidity of HCl introduced into the cat's stomach decreased by a process which appeared to be ordinary diffusion. He also observed simultaneous outwardly directed diffusion of alkali chlorides from the mucosal cells or from the blood into the stomach content.

Davenport (1966) using dogs showed that acid in the gastric lumen diffuses slowly through the normal mucosa, the rate depending on the natural permeability of the mucosa. Acid diffuses much more rapidly

through the mucosa whose permeability has been increased by damage, and it causes liberation of histamine into interstitial fluid. He showed that this histamine further increases the permeability of mucosal capillaries with the result that plasma proteins enter the interstitial fluid in abnormal amounts. Because mucosal permeability is increased, interstitial fluid enters the lumen. Davenport (1966) analysed this fluid and showed that it is not simply an ultrafiltrate of plasma. It has high Na^+ , K^+ and Cl^- contents. The fluid also contained protein. Fluid entering the lumen neutralizes and dilutes acid. Histamine and other humoral agents do not in themselves cause capillary rupture, but acid rapidly diffusing through the mucosa does. Interstitial hemorrhages and appearance of blood follow.

Cooke and Kienzie (1974) studied the effects of acetylsalicylic acid on the gastric mucosa of the dog. There was a net gain of Na^+ into the lumen and loss of H^+ from the lumen in response to aspirin. There has been no study on the biochemical reaction of capsaicine and Capsicum in the stomach.

Capsicum and capsaicine have been observed to be strong irritants on parts of the gastrointestinal tract. Very few studies have been done on the changes of the gastric mucosa by these substances. Other irritating substances have been shown to influence the biochemical response of the stomach. It was felt to be of interest to study the changes of the gastric mucosa to Capsicum and capsaicine by light and electron microscopy. In addition, the biochemical response of the stomach to these substances was observed. It is hoped that some correlation may be found between the morphological response to the mucosa of the stomach and its biochemical reaction to Capsicum and capsaicine.

MATERIALS AND METHODS

Ninety three albino rats purchased from Locke-Erickson Laboratories were used for this investigation. The rats, weighing 300-400 grams, included males and females of breeding age. All animals had available ad libitum, water and rat chow and were housed in an air conditioned animal room which was illuminated on a 12 hour light and 12 hour dark cycle. This investigation was conducted in two ways:

1. A long term phase in which rats were fed, in addition to their ad libitum diet, by oral intubation either an aqueous extract of red hot pepper (hence forth referred to as Capsicum), or a 5% ethyl alcoholic solution of capsaicine (the active principle of red hot pepper) for periods of several months.
2. A short term phase in which the stomach was opened surgically and the gastric mucosa of the emptied stomach was exposed to a 5% ethyl alcoholic solution of capsaicine for periods up to 90 minutes duration. The details of administration and preparation for these two phases will be presented subsequently along with provision for appropriate control solution administration.

PREPARATION OF SOLUTION:

The Capsicum extract preparation was begun by triturating 600 gms. of red pepper (*Capsicum annum*) in an Osterizer blender and run at low speed. The coarsely ground pepper (*Capsicum annum*) was placed in an Osterizer blender and run at high speed for 5 minutes. It was subsequently placed in a homogenizer and run at high speed for 5 to 10 minutes. This was suspended in 1000 ml of distilled water, heated to boiling, and

decanted. The sediment-free solution was filtered while hot and allowed to cool. Three Kg of Capsicum yields 1-1.5 gm of capsaicine (Toh et al, 1955; Kosuge et al, 1958). Three ml of this solution was given to each female rat (weighing in the range of 300 gm) daily and four ml to each male rat (weighing in the range of 400 gm). Each ml contained approximately 0.3 mg of capsaicine.

The capsaicine for oral feeding was made by dissolving 0.3 gm of capsaicine in 125 ml of 40% ethyl alcohol and distilled water was added to bring the solution to 1000 ml. Capsaicine is slightly soluble in cold water. Three ml of this solution was given to each female rat and four ml to each male rat daily for three to nine months.

The amount of capsaicine (1 mg/Kg) administered to the rats in this study is approximately equivalent to that contained in average single meal of the rural Thai people (Interdepartmental Committee on Nutrition for National Defense, 1962). The burning sensation of capsaicine, 1 part/100,000 can be detected by tasting (I.C.N.N.D., 1962).

LONG TERM PHASE:

Seventy two rats were used for this phase of the investigation. The rats were fed orally with solutions of capsaicine or Capsicum (red hot pepper extract) by feeding tube daily for three to nine months. For these long term studies, the rats were divided into the following groups:

Group I - This group consisted of twelve male rats fed orally with red hot pepper extract. Three rats were sacrificed at the end of three months, three at the end of six months and four rats at the end of nine months. There were two fatalities in this group: one died after one month and the other after four months of receiving red pepper extract.

Group II - This group consisted of six male rats to serve as control for Group I. They were intubated with water for the same length of time as those in Group I. Two rats were sacrificed at the end of three months, two at the end of six months and two at the end of nine months.

Group III - This group consisted of twelve female rats fed with red hot pepper extract. Three rats were sacrificed at the end of three months, three at the end of six months and two at the end of nine months. There were four fatalities in this group: one died after one month, one died the second month, and two died after the fourth month of receiving test solution.

Group IV - This group consisted of six female rats to serve as control for Group III. They were intubated with water. Two rats were sacrificed at the end of three months, two at the end of six months and two at the end of nine months.

Group V - This group consisted of twelve male rats fed with capsaicine solution (capsaicine in 5% ethanol). Three rats were sacrificed at the end of three months, three at the end of six months and three at the end of nine months. There were three fatalities in this group: one died after two weeks and two died after three months of receiving test solution.

Group VI - This group consisted of six male rats fed with 5% alcohol as control for Group V. Two rats were sacrificed at the end of three months, two at the end of six months and two at the end of nine months.

Group VII - This group consisted of twelve female rats fed with a 5% alcoholic solution of capsaicine. Three rats from this group were sacrificed at the end of three months, three at the end of six months and two at the end of nine months. There were four fatalities in this group: one died after two weeks, one after three months and two after four months of receiving the test solution.

Group VIII - This group consisted of six female rats fed with 5% alcohol. This acted as the control for Group VII. There was one fatality in this group caused by mistakingly emptying the test solution into the trachea instead of the stomach.

A total number of fourteen rats died during the entire period of the experiment. Six rats died from the groups fed with Capsicum (red pepper) extract, seven from capsaicine-treated groups and one from the control group.

Feeding needles (Fig. 2) obtained from Popper & Sons, Inc., New York, were used for oral feeding. To keep the rat in a good position and to avoid strangling the rat during feeding, a special feeding bag (Fig. 2), opened at both ends, was used. At the end of each experimental period (three months, six months or nine months), the rats were sacrificed by ether on the day after the last administration of the test substance. The abdominal cavity was rapidly entered and the stomach opened by a longitudinal incision. The stomach was examined grossly, photographed and processed for light microscopy.

For morphological studies, the stomach was fixed for twenty four hours in neutral buffered 10% formalin at room temperature and embedded in the conventional paraffin technique. Paraffin sections (7 μ) were

stained with hematoxylin and eosin (H & E).

SHORT TERM PHASE:

Twenty one rats were used for short term studies. For this investigation, rats were arranged into the following groups:

Group I - Five rats irrigated with a solution of capsaicine (in 5% alcohol) for 30 minutes.

Group II - Two rats irrigated with 5% alcohol for 30 minutes to serve as control for Group I.

Group III - Five rats irrigated with a solution of capsaicine (in 5% alcohol) for 60 minutes.

Group IV - Two rats irrigated with 5% alcohol for 60 minutes to serve as control for Group III.

Group V - Five rats irrigated with a solution of capsaicine (in 5% alcohol) for 90 minutes.

Group VI - Two rats irrigated with 5% alcohol for 90 minutes to serve as control for Group V.

The solutions given to control animals for any given experiment were always identical to the experimental solutions with respect to volume, solvent, and electrolyte composition. The only difference being that the capsaicine was omitted from the control solutions.

Before each test, the rats were deprived of food for twenty four hours. The test solution was made by dissolving .3 gm of capsaicine in 125 ml of 40% ethyl alcohol and distilled water added to bring the solution to 1000 ml. Three milliliters of the solution was given to each female rat and 4 ml to each male rat. The rats were anesthetised

with nembutal (.1 ml/100 gm of body weight). Each rat was placed on its back in a plexiglas (Lucite) box opened at the top (Fig. 14). A midline longitudinal incision was made along the greater curvature of the stomach. The cut edges of the stomach were drawn through a circular opening (2.5 cm in diameter) in a horizontal plastic sheet. A second plastic sheet was pressed against the first which served to hold the cut edges of the stomach maintaining a patent opening of the stomach. Two plates were held together by screws on either side of the opening of the stomach. Ligatures were tied around the pyloric and the cardiac openings of the stomach. The stomach was thoroughly rinsed with saline solution at the beginning of the experimental period. At the beginning of each experiment, the pouch was rinsed thoroughly with a saline solution (9 gm NaCl in 1000 ml of distilled water). The pouch was then drained and gently aspirated with a syringe. The test solution was drawn up into a syringe and the measured amount emptied into the pouch of the stomach and thoroughly mixed. Then, an approximately 1.0 ml sample was immediately withdrawn and saved for analysis. The instant of removing this sample was counted as the beginning of a period of observation. During the observation period, the contents of the pouch was frequently mixed by drawing some of the material in and out of the syringe. At the end of each experimental period, the pouch was aspirated as completely as possible. This final sample was saved for analysis.

The amount of sodium, potassium, chloride and acid entering and leaving during these periods were calculated as the difference between the concentration initially and finally present.

By convention, net losses (absorption) or one way fluxes from pouch to animal (insorption) were reported as negative quantities and net gains (enterosorption) or one way fluxes from animal to pouch (exsorption) as positive ones. All values for electrolytes were reported as milliequivalent per period of observation. Sodium and potassium were measured with a direct reading from automated flame photometer (Instrumentation Laboratory, Inc. 343) and each sample was bracketed with appropriate standards 140 mEq. Na/5mEq. K. and read against lithium (internal standard) before using. Chloride was measured with a chloride meter (Corning 920M). Acidity was calculated from pH meter (Corning, 12) readings. Hemoglobin analysis was done in the Loyola University Medical Center Clinical Chemistry Laboratory. All values for electrolytes are reported as milliequivalents per period. The data collected from these analyses were subjected to statistical analysis to determine whether there were significant differences between animals irrigated with capsaicine and the control animals. Differences between control data and those of the experimental were evaluated by the t-test. Differences were also calculated between data from 30 minutes and 60 minutes, 30 minutes and 90 minutes, 60 minutes and 90 minutes exposures. Significant differences in this context mean that P was less than 0.05.

For morphological studies, the rats were sacrificed at the end of each experimental period and the stomach fixed for light, scanning and transmission electron microscopic studies. This was done by bisecting the stomach longitudinally; one half fixed for light microscopy and the other half for scanning and transmission electron microscopy. No effort was made to remove the adherent mucous layer.

For light microscopy, the stomach was fixed for twenty four hours in neutral buffered 10% formalin at room temperature and embedded by the conventional paraffin technique. Paraffin sections (7 u) were stained with hematoxylin and eosin (H & E) or with alcian blue and periodic acid Schiff (PAS). The sections were examined under a regular compound light microscope (A. O. Spencer, 10).

For scanning electron microscopy, tissue cubes were cleared in amyl acetate and dried in Denton critical-point drying apparatus, using liquid carbon dioxide. Specimens were mounted on aluminum stubs, coated with gold and examined with an ISI-Mini scanning electron microscope operated at 15 KV.

For transmission electron microscopy, a 2 mm strip of the tissue was immediately placed into a cold buffered 3% Gluteraldehyde (Sabatini et al, 1963), cut into small pieces measuring 1 x 1 x 5 mm each. After three hours in osmium tetroxide (Palade, 1952) dehydrated in alcohol and washed in propylene oxide, tissues were embedded in epon (Luft, 1961) and allowed to polymerize at 56°C for forty eight hours. Epon blocks were trimmed to sizes of less than 1 x 1 mm under dissecting microscope.

Thick (1 u) and ultrathin (700 A) sections were cut with glass knife or diamond knife for light and transmission electron microscopy respectively. The thick sections were stained with 1% aqueous solution of toluidine blue. The ultrathin sections were stained with lead citrate (Reynolds, 1963) and Uranyl acetate (Watson, 1958). Thin sections were examined with an RCA-EMU3H transmission electron microscope operated

at 50 KV. SEM specimens were also embedded in epon and examined similarly. The ultrastructure of these specimens was identical to that of material processed for TEM alone.

The specimens prepared for microscopic study were examined and differences noted between experimental groups and appropriate control groups as well as between experimental groups of different duration. Attention was given to the mucosal layer of the stomach with particular reference to:

1. Presence of superficial mucous layer.
2. Nature of the lining epithelium and glands.
3. Capillaries and the connective tissue of the lamina propria.
4. Cytoplasmic organelles and the inclusions.
5. Nuclear configuration.

EXPERIMENTAL RESULTS

Attention was devoted to the morphological appearance of the gastric mucosa: its epithelial cells and lamina propria as observed grossly, by light, scanning, and transmission electron microscopy. In addition, the concentrations of sodium, potassium, chloride and hemoglobin in the gastric contents were measured before and after each experimental period.

LONG TERM PHASE (Intubation)

Gross and microscopic observations on male and female animals are presented together, as there were no sex differences in tissue reaction to Capsicum or capsaicine or the vehicular controls.

GROSS OBSERVATION:

All animals of capsaicine or Capsicum groups had loose mucous stools during the first week of long term feeding. Loose stools occurred intermittently in some of these animals during the remaining weeks of this investigation. Nearly all the experimental animals had at some time, loose bloody stools. Immediately on feeding with the test substance, the animals showed temporary signs of distress and a rise in rectal temperature (Fig. 51). A total of 22 rats vomited dark reddish blood 5 minutes after oral feeding with Capsicum or capsaicine for the first time. No vomitting of blood was observed in the control animals. Fresh blood was observed on the gastric mucosal surface of all capsaicine and Capsicum-treated rats (Fig. 5). Capsaicine and Capsicum solution feeding gave quite similar results except where stated (as in temperature, Fig. 51), hence their results are given together.

LIGHT MICROSCOPY:

Three Months Intubation: The animals receiving the control solutions, i.e., water (Fig. 8) or 5% alcohol (Fig. 8a) showed no alteration from the normal rat gastric mucosal pattern. The mucosal cells and glands were intact. In animals receiving red hot pepper extract or capsaicine solution, the blood vessels were dilated and some were exposed to the lumen by the loss of lining epithelium (Fig. 9). Some capillaries were completely disrupted and there was associated loss of red blood cells into the adjacent interstitial areas and into the lumen (Fig. 9a).

Six Months Intubation: Figures 12 and 12a show rat stomach fed with water or 5% alcohol respectively (controls). In both cases, the gastric mucosa is intact with normal glands and foveal. In rats receiving either red pepper extract (Fig. 13) or capsaicine solution (Fig. 13a), there was acute inflammation, hyperemia, glandular degeneration and intertubular edema. Some capillaries had ruptured and some had been exposed to the lumen by loss of lining epithelium. In some animals, the gastric glands were dilated (Fig. 13). Lesions presented active hyperemia limited to the mucosa of the stomach coupled with inflammatory reaction involving mucous cell layer (Fig. 13).

The capillaries of the lamina propria of rats in all experimental groups were swollen and congested (Figs. 9, 11 and 13). All the experimental animals exhibited pathological changes ranging from edema, hyperemia, congestion, hemorrhages to overt or frank bleeding.

Nine Months Intubation: The rat stomach fed with test substances had all the histopathologic changes observed in six months rat, in

addition the lamina propria of rats fed with the test substance for nine months, there was an increase in the number of lymphocytes, eosinophils, neutrophils, and plasma cells.

Generally, the remainder of gastric mucous cells away from an erosion area, appeared morphologically similar to those in tissue from control animals (Fig. 9). Some blood vessels were ruptured and some were extensively dilated. The most pronounced changes were seen in groups in which the animals were fed for nine months with test substance (Figs. 7 & 13).

Figure 13b shows morphological changes observed in long term rats intubated with Capsicum or capsaicine and their water or 5% ethyl alcohol controls for three, six or nine months. All the experimental rats in all the experimental periods had gastric content stained with blood and all had some degree of damage on the gastric mucosa. The submucosa of experimental rats from three months was normal. Five rats and eleven rats from six and nine months experimental periods, respectively had a severely damaged submucosa with an increased number of lymphocytes, eosinophils, neutrophils and plasma cells. Four rats from six months and eleven from nine months had dilated gastric glands. The control rats remained normal throughout all the periods of observation.

SHORT TERM PHASE

LIGHT MICROSCOPY:

Figures 15 and 20 are light micrographs of rat's stomach exposed to capsaicine for 30 minutes, 60 minutes and 90 minutes. Control figures are also shown for comparison. Noticably injured surface mucous cells were readily distinguishable in a 1.0 u thick epon section stained

with toluidine blue (Fig. 18), and damaged areas were found in all stomachs which received brief exposure to capsaicine.

30 Minutes Exposure to Capsaicine or 5% Alcohol: In the gastric mucosa exposed to the control solution of 5% alcohol, the gastric glands and cells were normal; the epithelium of the luminal surface was intact (Fig. 15). Figure 16 is the rat stomach exposed to capsaicine for 30 minutes. Alcian blue and periodic acid Schiff (PAS) were used to stain this section for the purpose of identifying the mucinogen granules. The presence of these granules is characteristic of normal mucous cells. Alcian blue stains the acid mucopolysaccharide part of the granules and PAS stains the neutral part. Some areas of the mucosa are necrotic. In Figure 16a, which was taken from the same specimen as in Figure 16, the section was stained with Hematoxylin and Eosin (H & E). The capillaries were dilated and congested with red blood cells. Some of the capillaries were exposed to the luminal surface and some were ruptured.

60 Minutes Exposure to Capsaicine or 5% Alcohol: The animals receiving the control solution of 5% alcohol showed no alteration from the normal rat gastric mucosal pattern. The mucosal cells and glands were intact (Fig. 17). In Figure 17a, which came from the same rat as in Figure 17, the section was 1u thick and stained with toluidine blue. The cell apices are smooth and rounded, characteristic of normal cells.

In rats exposed to capsaicine for 60 minutes, the surface epithelial cells were severely damaged and their destruction exposed dilated capillaries within whose lumen the red blood cells have hemolysed (Figs. 18 & 18a). Underlying glands, cells and deeper blood vessels showed no damage.

90 Minutes Exposure to Capsaicine or 5% Alcohol: In the gastric mucosa exposed to 5% alcohol for 90 minutes, the mucosal epithelium

was intact with normal glands and cells (Fig. 19). In Figure 20, in which the gastric mucosa was exposed to capsaicine for 90 minutes, there were some erosion in the gastric mucosa with some dilation and some exposure of blood vessels to the gastric lumen. Damaged cells were apparent as the underlying, partially denuded capillary is in open communication with the gastric lumen.

All the animals were affected by capsaicine. Ninety minutes exposure of the rat's stomach to capsaicine produced the greatest damage, 30 minutes the least and 60 minutes in-between the two. It is the epithelial surface lining which was mostly affected by the agent.

Superficial erosions of the mucosa were frequently encountered in stomachs exposed for 30, 60 and 90 minutes to capsaicine. The first cells to be lost were those on the free surface crests between the foveolar mouths; the damaged cells on the foveolar rims then followed. In Figures 16, 18 and 20, numerous sloughing and sloughed cells can be recognized. The ruptured mucous granules have disappeared from these cells, an observation which was confirmed at the ultrastructural level (Fig. 41). Only broken membrane remnants remained where the mucous granules formerly were localized. Some denuded capillaries congested with erythrocytes appeared relatively intact (Fig. 18), but the erythrocytes themselves showed definite variations in staining intensity not evident in control animals. These variations probably represent the early stages of intravascular hemolysis, an interpretation supported by Figure 35, which shows a disintegrating capillary still ensheathed with connective tissue elements, within whose lumen are trapped a number of lysed erythrocytes. At this stage the gastric pits have been almost completely eroded. The parietal cells underlying these superficial erosions seem to have remained unaltered by the capsaicine (Figs. 16, 18 and 20).

SCANNING ELECTRON MICROSCOPY:

Examination by scanning electron microscopy of the gastric surface of all control rats revealed the normal mucosal surface convolutions and epithelial cells (Figs. 23, 27 & 30). The presence of mucous on the surface of the cells (Fig. 23), which usually obscured the identity of the cells, gave evidence to the normal integrity of these cells. Differences related to varying duration of exposure to capsaicine prior to sacrifice (30 to 90 minutes) could be detected. Lesions ranged from breaks in the cells to cavitations in the surface cells.

Topographical examination of gastric epithelia revealed significant destruction of the superficial cells (Figs. 25, 29 & 32). The surface luminal integrity was greatly eroded. The erosion increased in intensity in proportion to length of exposure to the test material. The region showed in Figures 21 & 27 was typical of the normal fundic morphology. Foveolae are numerous in the rat and are typically separated by a distance equal to the diameters of three to five surface epithelial cells. Surface epithelial cells (SEC) are slightly convex and tightly abutted (Fig. 21).

Exposure of the gastric lumen to capsaicine resulted in extensive apical damage. Some regions showed large numbers of severely eroded cells (Fig. 32). Damage was widespread, although many cells were still unaffected. Even comparatively unaffected regions did, however, contain foci of degenerating cells (Fig. 25). In some areas, the surface epithelium was totally absent, exposing the underlying tissue and gland structure (Figs. 29 & 32).

It was observed that luminal surface integrity is a poor indication of normal intracellular morphology. It became imperative that extensive transmission electron microscopy be carried out in conjunction with topographical studies if in-depth understanding of the pathological changes is to be achieved.

TRANSMISSION ELECTRON MICROSCOPY:

The increase in the number of apically eroded and degenerating superficial epithelial cells seen with the scanning electron microscope was also visible with the transmission electron microscope: extensive cellular pathology. Such cells are characterized by karyolysis and margination of chromatin, dissolution of cytoplasmic organelles and absence of microvilli.

Figures 41, 43 and 45 are electron micrographs of capsaicine-damaged epithelial cells. The figures are arranged in sequence, beginning with the earliest (30 minutes) signs of intracellular disturbances, and ending with examples of cell lysis (90 minutes).

30 Minutes Exposure to Capsaicine or 5% Alcohol: Figure 40 is a transmission electron micrograph of rat gastric mucosa; mostly surface mucous cells on the luminal surface exposed to 5% alcohol for 30 minutes. Some features of cellular fine structure are shown. The mucous granules and junctional complexes are intact. In the gastric mucosa exposed to capsaicine for 30 minutes, some of the cells were severely damaged with extensive cytoplasmic swelling (Fig. 41). Some mucous granules were disrupted while others were relatively intact. The plasma membrane seemed to be intact.

60 Minutes Exposure to Capsaicine and 5% Alcohol: Normal clustering of mucous granules in the apical aspect of the cells were evident in control rats exposed to 5% alcohol (Fig. 42). The nucleus and cytoplasmic organelles were normal. Figure 43 is a transmission electron micrograph of capsaicine for 60 minutes. The surface of the cell was disrupted. There were breaks in the plasma membrane. Some of the mucous granules lost their contents and only the ruptured enclosing membrane remained.

90 Minutes Exposure to Capsaicine and 5% Alcohol: The gastric mucosal cells exposed to 5% alcohol for 90 minutes were similar to those exposed for 30 or 60 minutes. The cellular fine structures were intact: mucous granules and microvilli were normal (Fig. 44). In contrast to the control specimens, gastric mucosa exposed to capsaicine for 90 minutes was severely damaged (Fig. 45). The density of the cytoplasmic matrix in some of the cells was diminished (Fig. 47). Undulations developed in the apical cell periphery with increased separation of mucous granules (Fig. 47). Clumping of nuclear chromatin was much more pronounced (Fig. 48) than in control specimens.

Sizable membrane-bound vacoules were formed in the subnuclear cytoplasm of some cells (Fig. 48). In Figure 49, extensive damage was evident to the extent where positive identification of cell types was difficult to ascertain. Mitochondria were either damaged or swollen.

All experimental tissues showed some degree of damage by the test substance. Whole cells were swollen and disrupted; marked changes in nuclear morphology accompanied cytoplasmic alterations. The nucleus

enlarged and its chromatin became clumped and marginated (Fig. 48). The staining intensity of the cytoplasmic matrix diminished, resulting in heightened contrast between structural elements and the matrix. The cytoplasm thus took on a distinctly mottled appearance. The mucous granules in the terminal web became less uniformly clustered and coincidentally, in many cells, the free apical cell surface became distorted, with blebs of cytoplasm "ballooning" from the cell. Scattered mucous granules were caught up within these blebs (Fig. 49). Large vacuoles, containing a finely textured, very lightly stained substance, occasionally developed within or between damaged cells. These vacuoles were often close to the nucleus, deforming its normal contour (Fig. 48). All of these changes were observed in cells still attached to the gastric epithelium. When damage was more extensive, the cells were desquamated into the gastric cavity. These shed surface mucous cells were often preserved in our preparations, apparently trapped in the mucous secretions overlying the surface epithelium.

Cellular pathology was detectable within 30 minutes of instillation of the experimental solution. For this study, the optimal experimental time period was 90 minutes. By this time, damaged areas were found in all the mucosa, though with considerable individual variations in their frequency and extent. Damaged surface mucous cells in all stages of intracellular disarray lined such areas, but were often restricted to the free surface. There was also a distinct increase in the severity of surface disruption along the tips of the rugal crests. It is thus evident that the distribution of surface damage was patchy under these

experimental conditions, and that substantial areas of the mucosal surface escaped injury.

The changes within the nucleus were the simplest and most reliable index of the degree of injury to the cell. Reaggregation and redistribution of the chromatin were among the indications of damage; the nuclear sap also became more coarsely aggregated. The chromatin was much more conspicuous than in the normal nucleus. The nucleus gradually lost its structural identity.

The density of the ground substance progressively decreased, beginning at the same time as the changes in nuclear chromatin. Though the mucous granules became less uniformly clustered, they were not morphologically altered unless their covering membranes were ruptured. Their content appeared to diffuse into the cytoplasm at this point (Figs. 49 & 50). Mitochondrial swelling, if present at all, was minor in early and intermediate stages of cell damage, but in the later stages there was a variable amount of swelling in the matrix compartment (Fig. 50).

Cytoplasmic vacuolation was not visible within all surface mucous cells damaged by capsaicine, but it was striking in those cells or cell clusters in which it did occur. These vacuoles most often were in the perinuclear zone or in the expanded terminal web area of the injured cells (Fig. 48). The perinuclear vacuoles were membrane-bound and contained a randomly dispersed flocculent material similar in appearance to the extra-cellular substance seen often in the lumens of capillaries and between epithelial cells. The origin of the vacuoles whether from invaginations of the lateral cell membranes, or from the lysosome-like

sacs, or by de novo vacuolization - was not established in this study.

Pronounced distortions developed in the apical periphery of the damaged surface mucous cells. Large apical blebs with bizarre undulations and/or constrictions (Fig. 50), were not uncommon. Breaks in the plasma-lemmae were not observed until the process of cell degradation was far along (Fig. 41). On the lateral surface, the junctional complexes and desmosomal contacts remained intact even between moderately damaged cells (Fig. 41). In more severely damaged cells, aggregations of cytoplasmic matrix material were often found associated with the cell attachments. The cells often lysed in situ and disruption of the cell junctions did not appear to be a primary effect of the drug.

In control animals, cross sections of epithelial cells revealed normal clustering of mucous granules in the apical aspect of the cells (Figs. 40, 42 and 44). These cells displayed their normal cytologic characteristics. The presence of microvilli is also typical of normal cells (Fig. 44).

Experimental tissues showed degenerative changes. Specifically, there was disappearance of microvilli and/or absence of apical mucous granules (Figs. 47 and 49). Some cells displayed more advanced degeneration with generalized, cellular disintegration (Fig. 50). Figure 50a shows the occurrence of morphological changes observed in short term rats exposed to capsaicine (test substance) or 5% ethyl alcohol (control) for 30, 60 or 90 minutes.

All the experimental rats from all groups extravasated blood when the gastric mucosa was exposed to capsaicine. Both scanning and transmission electron microscopy revealed the absence of microvilli in all experimental rats; and all lost some amount of mucinogen granules. Clumping of chromatin material was prominent in all rats exposed to capsaicine for 90 minutes, while only one rat exposed for 60 minutes showed this change. All 90 minute rats had perinuclear vacuoles, two 60 minute rats and none of the 30 minute rats showed this vacuolation.

BIOCHEMICAL ANALYSIS:

Apart from histological examination, injury to the mucosa is revealed by increased output of sodium, potassium and chloride and by increased disappearance of acid. The magnitude of the increase influxes of sodium, potassium and chloride and the disappearance of acid were the measure of the damage inflicted. The flux of these substances are a sensitive index of the integrity of the gastric mucosal barrier. It is low when the barrier is intact and it increases when the barrier is broken. Injury is also determined by the amount of hemoglobin in the gastric contents.

Each experiment involved a 30 minute, a 60 minute and a 90 minute control period in which the test solution used was 5% alcohol. All control data from all groups were pooled and are reported here. No changes were observed in the amounts of sodium, potassium and chloride nor in acidity. Samples of the test solution of capsaicine and 5% alcohol were analyzed for sodium, potassium, acidity and hemoglobin. Sodium, potassium and chloride contained in the gastric content at the end of each experimental period increased drastically (Figs. 52 to 58). The acidity (H ions) decreased rapidly following an experimental curve with a simultaneous rise on Na^+ , K^+ , and Cl^- (Fig. 58). The loss of

H^+ from the lumen was much greater than in the controls. The values reported in these figures are means \pm standard error. Na^+ , K^+ and Cl^- moved into the lumen. In the final periods, blood appeared in the test solution. In a few rats, bleeding was so alarming, that the experiment was suddenly stopped and another rat substituted.

During the periods of irrigation with capsaicine, there were very greatly increased fluxes of all ions and bleeding began. These are all signs of severe disruption of the mucosal barriers. No such changes occurred in control experiments in which 5% alcohol was used. The amount of hemoglobin increased drastically in proportion to length of exposure to capsaicine (Fig. 56).

All changes indicate that the mucosal barriers were disrupted by capsaicine (Fig. 60).

The property of the gastric mucosa which allows it to contain an acid solution and which prevents rapid penetration of itself by hydrogen ions is the gastric mucosal barrier. The ability of the mucosa to prevent rapid diffusion of ions from its interstitial space into the lumen is likewise a property of the gastric mucosal barrier. The normal mucosa is divided into several compartments by barriers having different degrees of permeability. In Figure 59 are shown the compartments and barriers based upon analysis of canine gastric mucosa (Davenport, 1964) and supported by studies of the frog gastric mucosa (Cohen, 1962) and of human gastric mucosa (Hogben, 1955).

In practice, measurement of ionic exchanges across the mucosa gives a reliable estimate of the integrity of the barrier and so does the measurement of disappearance of acid.

The gastric mucosal barrier is broken by many compounds; among them are acid - HCl in high enough concentration (about 300 mN) and aliphatic acids such as acetic, propionic and butyric acids, which, because they are un-ionized and fat soluble, diffuse through cell membranes. Other compounds breaking the barrier are detergents, natural (bile salts and lysolecithin) or synthetic (lauryl sulfate), ethanol in a concentration of about 10% by weight, salicylic and acetylsalicylic acids in acid solution but not in neutral solution, some phospholipases and some local anesthetics. Contact of all these compounds for the appropriate time and in the appropriate concentration,

with the gastric mucosa results in a large increase in mucosal permeability (Davenport, 1964; Hingson and Ito, 1971).

Acid, which diffuses slowly through the normal mucosa, rapidly enters one whose barrier has been broken. Acid destroys mucosal cells and liberates histamine (Davenport, 1967). Histamine stimulates acid secretion, causes vasodilatation and increases capillary permeability. The mucosa becomes edematous, and fluid derived from interstitial fluid is forced through the mucosa. This fluid may contain a large amount of ions and plasma proteins. Acid stimulates the intramural plexuses, and motility of the stomach is increased. It also stimulates pepsin secretion, perhaps by way of histamine liberation and perhaps through its effect of the plexuses. Mucosal capillaries may be destroyed by acid so that interstitial hemorrhage and frank bleeding occur. Bleeding is more frequent and copious when there is concurrent cholinergic stimulation, probably because contraction of gastric muscle increases venous and capillary pressures (Hingson and Ito, 1971).

Hydrogen ions move rapidly through the damaged barrier, and their flow is facilitated by exchange for sodium (Fig. 59). Entry by hydrogen ions into the mucosa exacerbates damage caused by other agents, and sequential destruction of the mucosa follows. Potassium and organic cell contents enter the lumen, followed by sodium and chloride from interstitial fluid. Upon further destruction of barriers, a fluid containing plasma protein flows from interstitial spaces and capillaries. If plasma proteins can move through the mucosa in one direction, pepsin ought to be able to move through it in the other (Davenport, 1967). Finally, after the most severe damage, bleeding occurs.

Davenport, who is always quoted, has done more work on the effects of cytotoxic substances on the G.I. tract than any one else. He has studied the effects of both organic and inorganic acids (1966), aspirin and alcohols (1967, 1968), on the biochemistry of gastric mucosa of different animals. In all cases he observed that when the gastric mucosa was damaged by cytotoxic substance there was an influxes of Na^+ , K^+ and Cl^- into the lumen with a simultaneous disappearance of acid. His findings are now used as an index for determining the extent of damage inflicted on gastric mucosa by cytotoxic substances. Our findings are in agreement with Davenport's findings. We also confirm his findings that the influxes of electrolytes into the lumen increased with duration of exposure.

Our findings are in agreement with those of Cooke and Kienzle(1974). They studied anti-inflammatory drugs and aliphatic alcohols on antral mucosa of the dog. There were increased influxes of Na^+ , K^+ and Cl^- into the gastric lumen in response to aspirin, phenylbutasone, indomethacin and cortisone acetate, as well as the aliphatic alcohols of carbon chain lengths C_1 to C_{14} .

Our observations (Biochemistry) are not in agreement with the findings of Ketusingh et al.(1966) who studied the influence of Capsicum solution on gastric acidities. Four humans served as the subjects. The subjects swallowed Capsicum solution after which gastric juice was withdrawn at regular intervals until eight samples more had been collected. The samples were filtered and titrated against standard N/10 NaOH. Distilled water was used as the control. They report that after administration of Capsicum solution, the free acidity rose to and persisted at a higher level than water. The human subjects used in their investi-

gation were moderate users of Capsicum. Perhaps this observation may be a manifestation of a conditioned response or a species difference response.

Ultrastructural alterations in all capsaicine-treated rats were similar to those described in the studies of the cytotoxic effects of chemicals reported by a number of investigators. Svoboda et al (1966) and Hill et al (1968) found ultrastructural changes in the epithelial cells of the stomach in rats after long-term administration of low protein diets. Takano (1964), Freeman and Geer (1965) and Tandon et al (1969) have also described the sequence of ultrastructural alterations during experimental protein deficiency.

Our scanning and transmission electron microscopic findings are in agreement with the findings of Harding and Morris (1966). Harding and Morris conducted scanning and transmission electron microscopic studies on rat gastric mucosa exposed to aspirin. The authors observed loss of mucous granules. More granules were lost with longer exposures of the gastric mucosa to aspirin.

We also confirm the findings of Hingson and Ito (1971). The stomach of mouse was filled for 8 minutes by oral instillation of a 20mM solution of aspirin. The tissue was then fixed and prepared for electron microscopy. The surface epithelial cells were severely damaged and their destruction exposed swollen disintegrating capillaries. Some unusual vacuoles were observed in the cytoplasm closed to the nucleus, and there was a clumping of nuclear material.

We observed the enlargement of mitochondria reported by Nopanitaya and Nye (1974). Nopanitaya and Nye exposed rat duodenum to capsaicine solution and observed that some of the mitochondria in the experimental cells were either damaged or swollen. Both natural and synthetic capsaicine administered by the same route for the same length of time produced identical morphological alterations. Injury increased with duration of exposure, a finding confirmed by the present study.

Injury to the mucosa by Capsicum and capsaicine is revealed by light, scanning and transmission electron microscopical studies. Injury is also revealed by increased disappearance of acid. The magnitude of the increase influxes of these substances was the measure of the damage inflicted. This description leaves many things unexplained. We know very little about the intimate nature of the barriers, their physical and chemical structure, and their metabolic support. "Injury" itself is a vague term which covers events ranging from the most delicate molecular rearrangements to the most gross destruction of tissue. There were local differences in susceptibility to injury, and there were certainly differences from animal to animal. We know nothing of the chemical events underlying repair and recovery. These problems can all be attacked experimentally, and we hope that by presenting superficial descriptions of chemical injury, additional study will be provoked.

A study of the effects of capsaicine indicates the active principle of hot red pepper has great cytotoxic activity. Both natural and synthetic capsaicine have the same toxic effect. Capsaicine is the most stable active chemical in dried Capsicum pepper that can be extracted by simply boiling the ground pepper in water. The other chemicals which might be extracted, together with capsaicine from

fresh pepper, have been degraded by drying the fresh fruit, thus leaving only stable capsaicine (Nelson and Dawson, 1923). The toxic effect of natural capsaicine on the gastric mucosal cells was identical to that of the synthetic one when given by the same route for the same period of time. In both cases, the severity of morphological alterations increased with the length of exposure. This investigation has collected enough evidence to show that capsaicine solution upon direct contact with gastric mucosa, can produce pathological changes ranging from edema, congestion, hemorrhage to overt or frank bleeding. With regard to effect of Capsicum upon the gastric mucosa as demonstrated herein, the exclusion or rather reduction from the diet of people all over the world should be recommended. In patients with hematemesis or melena of unknown etiology, the possibility of hemorrhagic gastritis induced by Capsicum should be borne in mind by every gastroenterologist.

It is unlikely that we can precisely place all of the sequential steps in the dynamic formation of the superficial ulcer associated with acute erosive gastritis which develops subsequent to administration of capsaicine and Capsicum. However, one series of progressive events which might be postulated from our collected data is as follows:

- Focal damage of epithelial cells (loss of cellular contents)

- Cell destruction

- Cell necrosis

- Epithelial sloughing

- Superficial ulcer formation

SUMMARY

The effects of Capsicum (hot pepper) and its active principle, capsaicine on the morphology and biochemistry of gastric mucosa was studied on albino rats. Some rats were fed with red hot pepper for three months, some for six months and others for nine months. This constituted the long term phase of this investigation. At the end of each period, the animals involved were sacrificed and the stomach prepared for light microscopic studies.

In rats fed with the test solution for three or six months, the damage was confined to the superficial mucosal cells and a few associated cells. In some rats fed for nine months, damage extended deep into the submucosa. The gastric contents in all the experimental animals were bloodstained. Some sloughing of mucosal cells occurred and the internal cytoplasm of the superficial mucous cells disrupted as a consequence of discharging large number of mucous granules. Dilation and rupture of subepithelial blood vessels were common in those areas affected by the test solution.

The animals for short term studies were anesthetised with nembutol and a midline longitudinal incision was made along the greater curvature of the stomach. Ligatures were tied round the pyloric and the cardiac openings of the stomach. The test solutions were drawn up into a syringe and expelled into the pouch of the stomach. The gastric contents were sampled at 30, 60 and 90 minute periods and saved for analysis. Damage to the mucosa was characterized in chemical as well as morphological terms. Chemical damage alters the mucosal barriers, allowing increased fluxes of ions across the mucosa. Desquamation and bleeding

accompany chemical damage. The effects obtained with whole fruits or crude extract of *Capsicum* and pure capsaicine solution can no doubt be attributed to capsaicine. Injury is also revealed by increased output of sodium, potassium and chloride ions and by increased disappearance of acid. The magnitude of the increase influxes of these substances was the measure of the damage inflicted.

All changes indicate that the mucosal barriers were disrupted by the hot pepper and its pungent principle, capsaicine. This investigation has shown that capsaicine damages the gastric mucosal barriers. Injury to the mucosa is revealed by light, scanning and transmission electron microscopical studies. Exposure of the rat's stomach to capsaicine produced a general increase in the incidence of damaged mitochondria. In control animals the glands are nice and orderly. The mucinogen granules are evenly distributed. In experimental animals, there was a loss of the granules; in some cases, complete absence of the mucinogen granules and microvilli. There were ruptured blood vessels with free blood cells. In some cases, there were complete break-down process.

Ultrastructural alterations in all capsaicine-treated and *Capsicum*-treated rats were similar to those described in the studies of the cytotoxic effects of other chemicals reported by a number of investigators (Davenport, 1964; Eastwood, 1975).

The present study of the effects of capsaicine indicates the pungent principle of hot red pepper has great cytotoxic activity. Both natural and synthetic capsaicine have the same toxic effect.

Capsaicine is the most stable active chemical in dried *Capsicum* pepper that can be extracted by simply boiling the ground pepper in water. The other chemicals which may be extracted together with capsaicine from fresh pepper have been degraded by the drying of the fruit, thus leaving only stable capsaicine (Nelson and Dawson, 1923). The toxic effect of natural capsaicine on the gastric mucosal cells was identical to that of the synthetic one when given by the same route for the same period of time. In both cases, the severity of morphological alterations increased with the length of exposure. This investigation has collected enough evidence to show that capsaicine solution upon direct contact with gastric mucosa, can produce pathological changes ranging from edema, congestion, hemorrhage, to overt or frank bleeding.

BIBLIOGRAPHY

- Allen, C.H., Gibbs, P.H. and Jersild, R.A. 1971. Intestinal fat transport during inhibition of protein synthesis. An electron microscopic study. *Exp. Mol. Pathol.* 14:90-102.
- Altamirano, M. 1969. Action of concentrated solution of non-electrolytes on the dog gastric mucosa. *Am. J. Physiol.* 216:33-40.
- Arstila, A.U. and Trump, B.F. 1968. Studies on cellular autophagocytosis. The formation of autophagic vacuoles in the liver after glucagon administration. *Amer. J. Pathol.* 53:687-733.
- Arstila, A.U. and Trump, B.F. 1969. Autophagocytosis: Origin of membrane and hydrolytic enzymes. *Virchow Arch. (Zellpath)*. 2:85-90.
- Astley, C.E. 1967. Gastritis, aspirin and alcohol. *Brit. Med. J.* 4:484.
- Augur, N.A., Jr. 1970. Gastric mucosal blood flow following damage by ethanol, acetic acid, or aspirin. *Gastroenterology*. 58:311-320.
- Babkin, B.P. 1950. *Secretory Mechanism of the Digestive Glands* (2nd ed: New York: Paul B. Hoeber, Inc.)
- Balcerzak, S.P., Lane, W.C. and Bullard, J.W. 1970. Surface structure of intestinal epithelium. *Gastroenterology*. 58:49-55.
- Barclay, A.E., Bentley, F.H. 1949. The vascularization of the human stomach-a preliminary note on the shunting effect of trauma. *Gastroenterology*. 12:177-183.
- Barlow, T.E., Bentley, F.H., Walder, D.N. 1951. Arteries, veins and arterio-venous anastomoses in the human stomach. *Surg. Gynecol. Obstet.* 93:657-671.
- Bevan, J.A. 1962. Action of lobeline and capsaicine on afferent endings in the pulmonary artery of the cat. *Cir. Res.* 10:792-797.

- Black, R.B., Hole, D., Rhodes, J. 1971. Bile damage to the gastric mucosal barrier: The influence of pH and bile acid concentration. *Gastroenterology*. 61:178-184.
- Bohr, D. and Goulet, P.L. 1961. Role of electrolytes in contractile machinery of vascular smooth muscle. *Amer. J. Cardiol.* 8:549-556.
- Bond, A.M. and Hunt, J.N. 1956. The effect of sodium fluoride on the output of some electrolytes from the gastric mucosa of cats. *J. Physiol.* 133:317.
- Briggs, A.H. and Melvin, S. 1961. Ion movements in isolated rabbit aortic strips. *Amer. J. Physiol.* 201:365-368.
- Brunser, O. and Luft, J.H. 1970. Fine structure of the apex of absorptive cells from rat small intestine. *J. Ultrastruct. Res.* 31:291-311.
- Bulkley, G., Goldman, H., Silen, W. 1969. Pressure injury to the gastric mucosa-studies on an in vivo model of acute gastric distention. *Am. J. Surg.* 117:193-203.
- Carson, F., Lynn, J.A. and Martin, J.H. 1972. Ultrastructural effect of various buffers, osmolarity, and temperature on paraformaldehyde fixation of the formed elements of blood and bone marrow. *Texas Rep. Biol. Med.* 30(2):125-142.
- Cheung, L.Y., Moody, F.G., and Reese, R.S. 1975. Effect of aspirin, bile salt and ethanol on canine gastric mucosal blood flow. *Surg.* 77(6):786-792.
- Clark, B.B. and Adams, W.L. 1947. The effect of acetylsalicylic acid on gastric secretion. *Gastroenterology*. 9:461-465.

- Code, C.F. 1937. The quantitative estimation of histamine in the blood. *J. Physiol. (London)*. 89:257-268.
- Code, C.F. 1956. Histamine and gastric secretion. p.189-219. In C.E. Wolstenholm and C.M. O'Connor (ed.) Ciba Foundation symposium on histamine. J. & A. Churchill Ltd., London.
- Code, C.F. 1965. Histamine and gastric secretion: a later look. *Fed. Proc.* 24:1311-1321.
- Code, C.F., Hallenbeck, G.A. and Gregory, R.A. 1947. Histamine content of canine gastric juice. *Amer. J. Physiol.* 151:593-605.
- Code, C.F., Higgins, J.A., Moll, J.C., Orvis, A.L., and Scholer, J.F. 1963. The influence of acid on the gastric absorption of water, sodium and potassium. *J. Physiol.* 166:110.
- Code, C.F., and McIntire, F.C. 1956. Quantitative determination of histamine. p. 49-95.
- Cohen, N., Horowitz, M.I., and Hollander, F. 1962. Serum albumin and gamma-globulin in normal human gastric juice. *Proc. Soc. Exp. Biol. Med.* 109:463-467.
- Coleridge, H.M. 1965. Pulmonary fibers stimulated by capsaicine. *J. Physiol. (London)*. 179:248-262.
- Coleridge, H.M., Coleridge, J.C.G., and Kidd, C. 1964. Role of the pulmonary arterial baroreceptors in the effects produced by capsaicine in the dog. *J. Physiol. (London)*. 170:272-285.
- Coleridge, H.M., Coleridge, J.C.G., and Luck, J.C. 1965. Pulmonary afferent fibers of small diameter stimulated by capsaicine and by hypoinflation of the lungs. *J. Physiol.* 179:248-262.

- Conrad, M.E., Crosby, W.H. 1963. Intestinal mucosa mechanism controlling iron absorption. *Blood*. 22:406-415.
- Cooke, A.R., and Kienzle, M.G. 1974. Studies of anti-inflammatory drugs and aliphatic alcohols on antra mucosa. *Gastroenterology*. 66:56-62.
- Curran, R.C., and Creamer, B. 1963. Ultrastructural changes in some disorders of the small intestine associated with malabsorption. *J. Pathol. Bacteriol.* 86:1-8.
- Davenport, H.W. 1943. The secretion of iodine by the gastric mucosa. *Gastroenterology*. 1:1055-1061.
- Davenport, H.W. 1962. Effect of ouabain on acid secretion and electrolyte content of frog gastric mucosa. *Proc. Soc. Exp. Biol. Med.* 110:613.
- Davenport, H.W. 1963. Sodium space and acid secretion in frog gastric mucosa. *Amer. J. Physiol.* 204:213.
- Davenport, H.W. 1963. Sodium and potassium in canine gastric mucosa and smooth muscle. *Amer. J. Physiol.* 205:413-416.
- Davenport, H.W. 1964. Gastric mucosal injury by fatty and acetylsalicylic acids. *Gastroenterology*. 46:245-253.
- Davenport, H.W., Warner, H.A., and Code, C.F. 1964. Functional significance of gastric mucosal barrier to sodium. *Gastroenterology*. 47:142-152.
- Davenport, H.W. 1965. Damage to the gastric mucosa: effects of salicylates and stimulation. *Gastroenterology*. 49:189-196.
- Davenport, H.W. 1965. Potassium fluxes across the resting and stimulated gastric mucosa: injury by salicylic and acetic acids. *Gastroenterology*. 49:238-245.

- Davenport, H.W. 1966. Fluid produced by the gastric mucosa during damage by acetic and salicylic acids. *Gastroenterology*. 50:487-499.
- Davenport, H.W. 1967. Ethenol damage to canine oxyntic glandular mucosa. *Exp. Biol. Med.* 126:657-662.
- Davenport, H.W. 1967. Salicylate damage to the gastric mucosal barrier. *N. Engl. J. Med.* 276:1307-1312.
- Davenport, H.W. 1967. Physiological structure of the gastric mucosa in Code, C.F. (ed.): *Handbook of Physiology: Sec. 6 Alimentary Canal, Vol. II* (Washington, D.C.:American Physiological Society).p.759-780.
- Davenport, H.W. 1968. Destruction of the gastric mucosal barrier by detergents and urea. *Gastroenterology*. 54:175-181.
- Davenport, H.W. 1972. Why the stomach does not digest itself. *Sci. Am.* 226:86-93.
- DenBesten, L., and Hamza, K.N. 1972. Effects of bile salts on ionic permeability of the canine gastric mucosa during experimental shock. *Gastroenterology*. 62:417-424.
- Eastwood, G.L. 1975. Effect of pH on bile salt injury to mouse gastric mucosa: a light and electron-microscopic study. *Gastroenterology*. 68(6):1456-1465.
- Eastwood, G.L., and Kirchner, J.P. 1974. Changes in the fine structure of mouse gastric epithelium produced by ethanol and urea. *Gastroenterology*. 67(1):71-84.
- Edlich, R.F., Borner, J.W., and Kuphal, J. et al. 1970. Gastric blood flow - 1. Its distribution during gastric distention. *Am. J. Surg.* 120:35-37.

- Estensen, R.D., and Baserga, R. 1966. Puromycin-induced necrosis of crypt cells of the small intestine of mouse. *J. Cell. Biol.* 30:13-22.
- Florey, H.W. 1960. Electron-microscopic observation of goblet cells of the rat's colon. *Quart. J. Exp. Physiol.* 45:329-336.
- Florey, H.W. 1962. The secretion and function of intestinal mucus. *Gastroenterology.* 43:326-329.
- Freeman, J.A., and Geer, J.C. 1965. Intestinal fat and iron transport, goblet cell, mucin secretion, and cellular changes in protein deficiency observed with electron microscope. *Am. J. Dig. Dis.* 10:1004-1023.
- Frenning, B., and Obrink, K.J. 1971. The effects of acetic and acetylsalicylic acids on the appearance of the gastric mucosal surface epithelium in the scanning electron microscope. *Scand. J. Gastrol.* 6(3):605-612.
- Geall, M.G., Phillips, S.F., and Summerskill, W.H. 1970. Profile of gastric potential difference in man. Effects of aspirin, alcohol, bile, and endogenous acid. *Gastroenterology.* 58:437-443.
- Glatzel, H. 1965. Spices and organ functions. Results of recent studies. *Muench. Med. Wchschr.* 107:332-342.
- Glatzel, H. et al. 1966. Radiologic studies on the effect of capsicum spice and mustard on the motility and secretion of the digestive tract. *Deutsch. Z. Verdau. Stoffwechsokr.* 26:113-123.
- Gregory, R.A. 1962. *Secretory Mechanisms of the Gastrointestinal Tract.* (London: Edward Arnold & Co.).
- Hally, A.D. 1959. The fine structure of gastric parietal cell of the mouse. *J. Anat.* 93:217.

- Hampton, J.C. 1967. The effects of nitrogen mustard on the intestinal epithelium of the mouse. *Radiat. Res.* 30:576-589.
- Hamza, K.N., and DenBesten, L. 1971. Bile salts producing stress ulcers during experimental shock. *Clin. Res.* 19:393.
- Harding, R.K., and Morris, G.P. 1966. Pathological effects of aspirin and hemorrhagic shock on the gastric mucosa of the rat. Proceedings of the workshop on advances in biomedical applications of SEM: IIT Research Institute, Chicago, Illinois.
- Helander, H.F. 1962. Ultrastructure of fundus glands of the mouse gastric mucosa. *J. Ultrastructure Research.* 4:
- Helander, H.F., and Eckhol, R. 1959. Ultrastructure of fundus glands of mouse gastric mucosa. *J. Ultrastructure. Research.* 3::74.
- Heubner, W. 1925. Further studies on the effects of undecylenacidic allinylamide. *Arch. Exp. Pharm.* 107:129.
- Hill, R.B., Jr., Prosper, J., and Hirschfield, J.S. 1968. Protein starvation and small intestine. 1. The growth and morphology of the small intestine in weaning rats. *Exp. Mol. Pathol.* 8:66-74.
- Hingson, D.J., and Ito, S. 1971. Effect of aspirin and related compounds on the fine structure of mouse gastric mucosa. *Gastroenterology.* 61:156-177.
- Hogben, C.A.M. 1954. Lactate transfer by isolated gastric mucosa: Theoretical consideration of weak electrolyte active transport. *Fed. Proc.* 13:71.
- Hogben, C.A.M. 1955. Active transport of chloride by isolated frog gastric epithelium; origin of the gastric mucosal potential. *Amer. J. Physiol.* 180:641.

Hogben, C.A.M., Schanker, L.S., Tocco, D.J., and Brodie, B.B. 1957.

Absorption of drugs from the stomach. II. Human. *J. Pharmacol. Exp. Ther.* 120:540.

Hollander, F., and Schapira, A. 1963. Gastric response to topical histamine; subcutaneous dose-response curve. *Amer. J. Physiol.* 205:625-630.

Hunt, J.N. 1954. The inhibitory action of sucrose on gastric digestive ability in patients with peptic ulcer. *Guys Hosp. Rep.* 103:161-173.

Hunt, J.N., and Wan, B. 1967. Electrolytes of mamalian gastric juice, in Code, C.F. (ed.) *Handbook of Physiology: Sec. 6. Alimentary Canal, Vol. II* (Washington, D.C.: American Physiological Society). p. 781-804.

Interdepartment Committee on Nutrition for National Defense: Nutrition Survey-The Kingdom of Thailand. Washington, D.C.: U. S. Government Printing Office, 1962. p. 57-59.

Ito, S. and Winchester, R.J. 1963. The fine structure of the gastric mucosa in the rat. *J. Cell. Biology.* 16:541-580.

Ivey, K.J. 1971. Acute hemorrhagic gastritis: modern concepts based on pathogenesis. *Gut.* 12:750-753.

Ivey, K.J., DenBesten, L., and Clifton, J.A. 1970. Effect of bile salts on ionic movement across the human gastric mucosa. *Gastroenterology.* 59:683-690.

Iwai, N. 1969. Histochemical studies of small and large bowels. Part 3. The effects of anti-tumor agents, colchicine and gamma-rays irradiation on gastrointestinal tracts of small laboratory animals. *Jap. Arch. Inter. Med.* 16:275-281.

- Jacobson, E.D. 1967. (ed.): Symposium on the gastrointestinal circulation. *Gastroenterology*. 52:328-471.
- Jacobson, E.D. 1967. Secretion and blood flow in the gastrointestinal tract, in Code, C.F. (ed.): *Handbook of Physiology: Sec. 6. Alimentary Canal*. Vol. II (Washington, D.C.:American Physiological Society. p. 1043-1062.
- Jacobson, E.D. 1968. Clearances of the gastric mucosa. *Gastroenterology*. 54:434-448.
- Jacobson, E.D., Chodos, R.B., and Falloon, W.W. 1960. An experimental malabsorption syndrome induced by neomycin. *Amer. J. Med.* 28:524-533.
- Jacobson, E.D., Lindford, R.H. and Grossman, M.I. 1966. Gastric secretion in relation to mucosal blood flow studied by a clearance technique. *J. Clin. Invest.* 45:1-13.
- Jacobson, E.D., Prior, J.T., and Falloon, W.W. 1950. Malabsorptive syndrome induced by neomycin: Morphologic alterations in the jejunal mucosa. *J. Lab. Clin. Med.* 56:245-250.
- Jansco, N., and Porszasz, J. 1959. Studies on the action potentials of sensory nerves in animals desensitized with capsaicine. *Acts. Physiol. Acad. Sci. (Hung.)*. 16:299-306.
- Jeffries, G.H. 1967. Gastric secretion of intrinsic factor, in Code, C.F. (ed.): *Handbook of Physiology: Sec. 6. Alimentary Canal*, Vol. II (Washington, D.C.:American Physiological Society. p. 919-924.
- Johnson, L.R. 1966. Histamine liberation by gastric mucosa of pylorus-ligated rats damaged by acetic or salicylic acids. *Proc. Soc. Exp. Biol. Med.* 121:384-386.

- Johnson, L.R., and Overholt, B.F., 1967. Release of histamine into gastric venous blood following injury by acetic or salicylic acid. *Gastroenterology*. 52:505-509.
- Joo, F., Szolcsanyi, J., and Jansco-Gabor, A. 1969. Mitochondrial alterations in the spinal ganglion cells of the rat accompanying the long lasting sensory disturbance induced by capsaicine. *Life. Sci.* 8:621-626.
- Kienzle, M.G., and Cooke, A.R. 1974. Studies of anti-inflammatory drugs and aliphatic alcohols on antral mucosa. *Gastroenterology*. 66:56-62.
- Ketusihn, O., Dhorranintra, B., and Juengjareon, K. 1966. Influence of capsaicine solution on gastric acidities. *Am.J. Proctol.* 17:511-515.
- Kosuge, S., Inagaki, Y., and Uehara, T. 1958. Studies on the pungent principles of Capsicum, 1. On the chemical constitution of the pungent principles. (1) On isolation of the pungent principles. *Nihon. Nougai Kagaku. Kaishi* (Japanese text). 32:578-581.
- Lagos Health Report (Nigeria) for 1972.
- Lee, S.O. 1963. Influence of diets and lipotropic substances upon the various organs and metabolic changes in rabbit on long term feeding with red pepper. Histopathologic changes of various organs. *Korea J. Int. Med.* 6:471-481. Reported by Ketushin et al in *Am. J. Proct.* 17:511-515.
- Lee, T.S. 1954. The pharmacological action of capsaicine and analogues. *Brit. J. Pharmacol.* 10:175-182.
- Levy, G., and Hayes, B.A. 1960. Physiochemical basis of the buffered acetylsalicylic acid controversy. *New Engl. J. Med.* 262:1053.

- Lillibridge, C.B. 1964. The fine structure of normal human mucosa. *Gastroenterology*. 47:269-312.
- Luft, J. 1961. Improvement in epoxy resin embedding methods. *J. Biophys. Biochem. Cytol.* 9:409-414.
- Menguy, R. 1972. Acute gastric mucosal bleeding. *Ann. Rev. Med.* 23:297-312.
- Millington, P.F., Fenean, J.B., Forbes, O.C., and Frazer, A.C. 1962. Studies of the effects of aminopterin on the small intestine of rats. I. The morphological changes following a single dose of aminopterin. *Expt. Cell. Res.* 28:162-178.
- Mólnar, J. 1968. Pulmonary hypertensive and other hemodynamic effects of capsaicine in cat. *Europ. J. Pharmacol.* 1:86-92.
- Morris, G.P., and Harding, R.K. 1974. Topography and fine structure of acute fundic mucosal erosions in the rat. *Lab. Invest.* 30(5):639-646.
- Nelson, E.K., and Dawson, L.E. 1923. The constitution of capsaicine, the pungent principle of capsicum. *J. Amer. Chem. Soc.* 45:2179.
- Nishino, N., and Fujiwara, M. 1969. Effect of capsaicine on the guinea-pig isolated atrium. *J. Pharm. Pharmacol.* 21:622-624.
- Nopanitaya, W. 1974. Effects of capsaicine in combination with diets of varying protein content on the duodenal absorptive cells of the rat. *Am. J. Dig. Dis.* 19:439-448.
- Nopanitaya, W., and Nye, S.W. 1974. Duodenal mucosal response to the pungent principle of hot pepper (capsaicine) in rat: Light and electron microscopic study. *J. Toxicology and Applied Pharmacol.* 30:149-161.
- Oi, M., Oshida, I., and Sugimara, S. 1959. The location of gastric ulcer. *Gastroenterology*. 36:45-56.

- Palade, G.E. 1952. A study of fixation for electron microscopy. *J. Exp. Med.* 95:285-297.
- Pease, D.C. 1964. *Histological Technique for Electron Microscopy*. 2nd ed. pp. 234-236. Academic Press, New York.
- Pfeiffer, C.J., and Weibel, J. 1973. The gastric mucosal response to acetylsalicylic acid in the ferret: an ultrastructural study. *Am. J. Dig. Dis.* 18(10):834-846.
- Porszasz, J., Gyorgy, L., and Porszasz-Gibisz, K. 1955. Cardiovascular and respiratory effects of capsaicine. *Acta. Physiol. Acad. Sci. Hung.* 8:61-76.
- Porszasz, J., Such, G., and Porszasz-Gibisz, K. 1957. Circulatory and respiratory chemoreflexes. I. Analysis of the site of action and receptor types of capsaicine. *Acta. Physiol. Acad. Sci. Hung.* 12:189-205.
- Porszasz, J., and Jamsco, N. 1959. Studies on the action potentials of sensory nerves in animals desensitized with capsaicine. *Acta. Physiol. Acad. Sci. Hung.* 16:299-306.
- Reynolds, E.S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell. Biol.* 17:208-212.
- Rhodes, J., Barnado, D.E., and Phillips, S.F., et al. 1969. Increased reflux of bile into the stomach in patients with gastric ulcer. *Gastroenterology.* 57:241-252.
- Roth, J.L.A., Valdes-Dapena, A., Pieses, P., and Buchman, E. 1963. Topical action of salicylates in gastrointestinal erosions and hemorrhage. *Gastroenterology.* 44:146.
- Rubin, E. 1971. Alcoholism, alcohol and drugs. *Science.* 172:1097.

- Ryback, B.J. 1962. Electron microscope studies of intestinal lesion.
I. Aminopterin-induced lesions in mice. *Gastroenterology*. 42:306-318.
- Sabatini, D.S., Bensch, K., and Barnett, R.J. 1963. The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. *J. Cell. Biol.* 17:19-58.
- Sirsat, S.M. and Khanolkar, V.R. 1960. Submucous fibrosis of the palate in diet-preconditioned Wistar rats. (Induction by local painting of capsaicine - An optical and electron microscope study). *Arch. Pathol.* 70:171-179.
- Skillmann, J.J., and Silen, W. 1970. Acute gastroduodenal ulceration: barrier disruption of varied pathogenesis. *Gastroenterology*. 59:478-481.
- Svoboda, D., Grady, H., and Higginson, J. 1966. The effect of chronic protein deficiency in rats: II. Biochemical and ultrastructural changes. *Lab. Invest.* 15:731-749.
- Takano, J. 1964. Intestinal changes in protein-deficient rats. *Exp. Mil. Pathol.* 3:224-231.
- Tandon, B.N., Newberne, P.M., and Young, V.R. 1969. A histochemical study of enzyme changes and ultrastructure of the jejunal mucosa in protein-depleted rats. *J. Nutr.* 99:519-530.
- Tegeris, A.S., Weide, G.V., and Curtis, J.M. 1968. Progressive ultrastructural changes in the mucosal epithelium of the small intestine of beagle dogs fed methoxychlor. *Exp. Mol. Pathol.* 8:243-247.
- Teorell, T. 1933. On the permeability of the stomach mucosa for acids and some other substances. *Skand. Arch. Physiol.* 66:225.

- Teorell, T. 1939. On the permeability of the stomach mucosa for acids and some other substances. *J. Gen. Physiol.* 23:263.
- Toda, N. 1971. Influence of cocaine and desipramine on the contractile response of isolated rabbit pulmonary arteries and aortae to transmural stimulation. *J. Pharmacol. Exp. Ther.* 179:198-206.
- Toda, N., et al. 1972. Cardiovascular effects of capsaicine in dogs and rabbits. *J. Pharmacol. Exp. Ther.* 181:512-521.
- Toda, N., Usui, H., and Mori, J. 1972. Contractile responses of spiral strips of some large blood vessels from rabbits to transmural stimulation, tyramine, dopamine and noradrenaline. *Jap. J. Pharmacol.* 22: 59-69.
- Toh, C.C., Lee, T.S., and Kiang, A.K. 1955. The pharmacological actions of capsaicine and analogues. *Brit. J. Pharmacol.* 10:175-182.
- Trier, J.S. 1962. Morphological alterations induced by methotrexate in the mucosa of human proximal intestine. II. Electron microscopic observations. *Gastroenterology.* 43:407-424.
- Trier, J.S. 1962. Morphologic alterations induced by methotrexate in the mucosa of human proximal intestine. II. Electron microscopic observations. 43:407-424.
- Trier, J.S. and Rubin, C.E. 1964. Electron microscopy of the gut: A word of caution. *Gastroenterology.* 47:313-315.
- Trier, J.S. and Rubin, C.E. 1965. Electron microscopy of the small intestine: A review. *Gastroenterology.* 49. 574-603.
- Varga, L. 1936. Action of various stimulants on gastric chemistry. *Orv. Hetil.* 80:702-704.

- Verbin, R.S., and Farber, E. 1967. Effect of cycloheximide on the cycle of the crypts of the small intestine of the rat. *J. Cell. Biol.* 35:649-658.
- Viranuvatti, V., Kalayasiri, C., Chearani, O., and Piengvanit, U. 1972. Effects of capsicum solution on human gastric mucosa as observed gastroscopically. *Am. J. Gastroenterology.* 58:225-232.
- Watson, M.L. 1958. Staining of tissue sections for EM with heavy metals. *J. Biophysiol. Biochemistry Cytology.* 4:475-478.
- Webb-Peploe, M.M., et al. 1972. Vascular responses to stimulation of receptors in muscle by capsaicine. *Am. J. Physiology.* 222:189-195.
- Weisbrodt, N.W., Kienzle, M., and Cooke, A.R. 1973. Comparative effects of aliphatic alcohols on the gastric mucosa. *Proc. Soc. Expt. Biol. Med.* 142:450-454.
- Werther, J.L., Janowitz, H.D., and Dyck, W.P. et al. 1970. The effect of bile on electrolyte movement across canine gastric antral and fundic mucosa. *Gastroenterology.* 59:691-697.
- Williams, A.W. 1963. Electron microscopic changes associated with water absorption in the jejunum. *Gut.* 4:1-7.
- Williams, A.W. 1963. Experimental production of altered jejunal mucosa. *J. Pathol. Bacteriol.* 85:467-472.
- Wolf, S. 1965. *The Stomach.* (New York: Oxford University Press).

Figure 1 is copied from Lagos Health
(Nigeria) Report for 1972.

Duodenal ulcer in black populations in Africa south of the Sahara

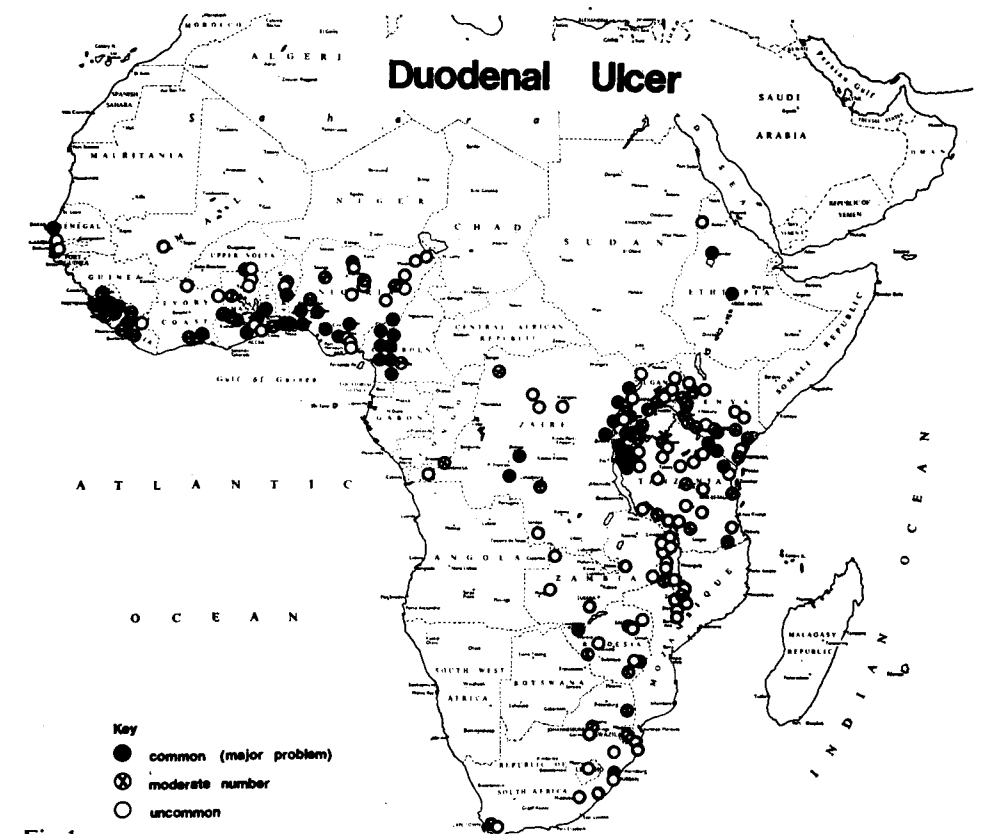


Fig 1

FIGURE 1

Figure 2 shows feeding bag and feeding needles.

Figure 2a shows x-ray of the rat with feeding needle in place.

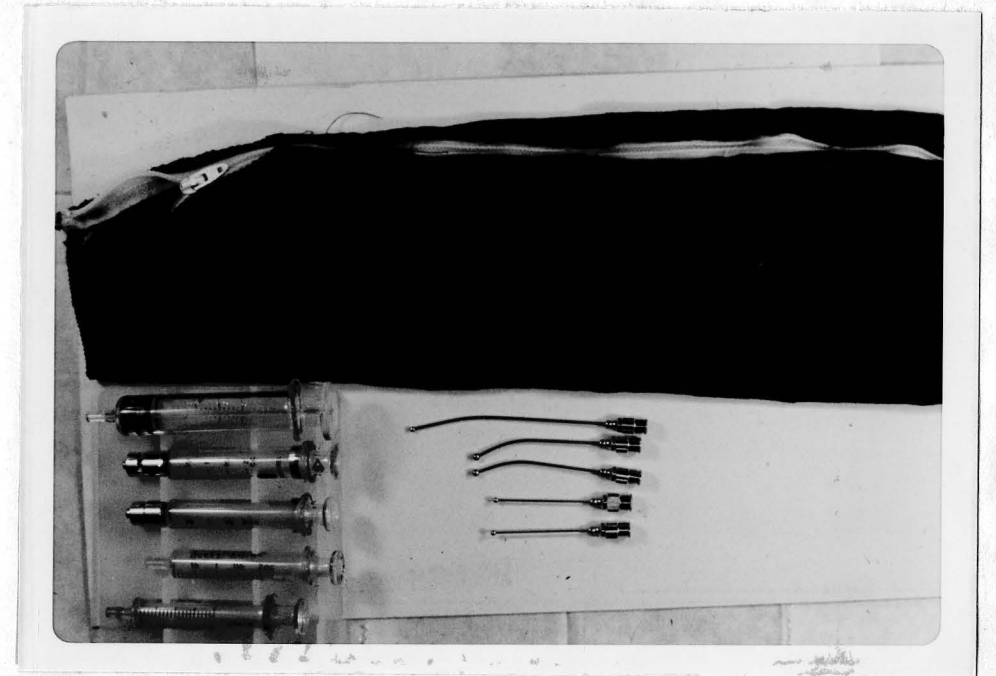


FIGURE 2

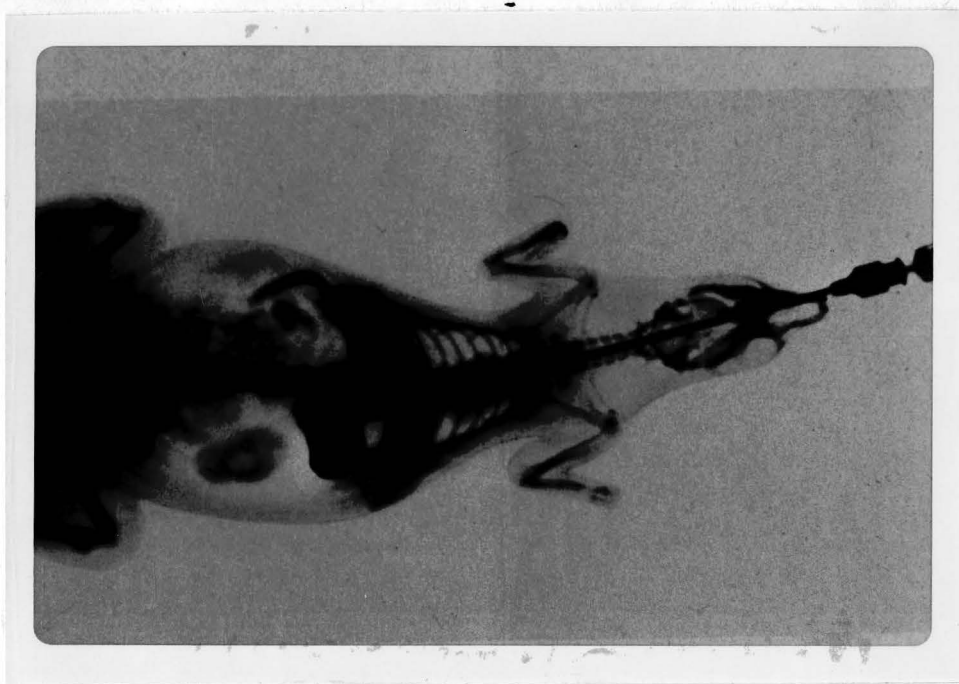


FIGURE 2a

Figure 3 shows technique for oral feeding.



FIGURE 3

Figure 4 shows the gross appearance of the rat gastric mucosa after 9 months of oral feeding with 5% alcohol (control). The darker area is the glandular portion of the stomach. The lighter translucent portion is the fore-stomach. The spleen is shown at the upper left.



FIGURE 4

Figure 5 shows the gross appearance of the rat gastric mucosa after 9 months of oral feeding with red hot pepper. Much of the glandular portion of the stomach is darkened by the presence of extravasated blood in the mucosal layer.



FIGURE 5

Figure 6 shows severe response of the mucosa to red hot pepper (9 months). This resulted in the "ballooning" of a portion of the gastric mucosa into the lumen (indicated by the arrow).

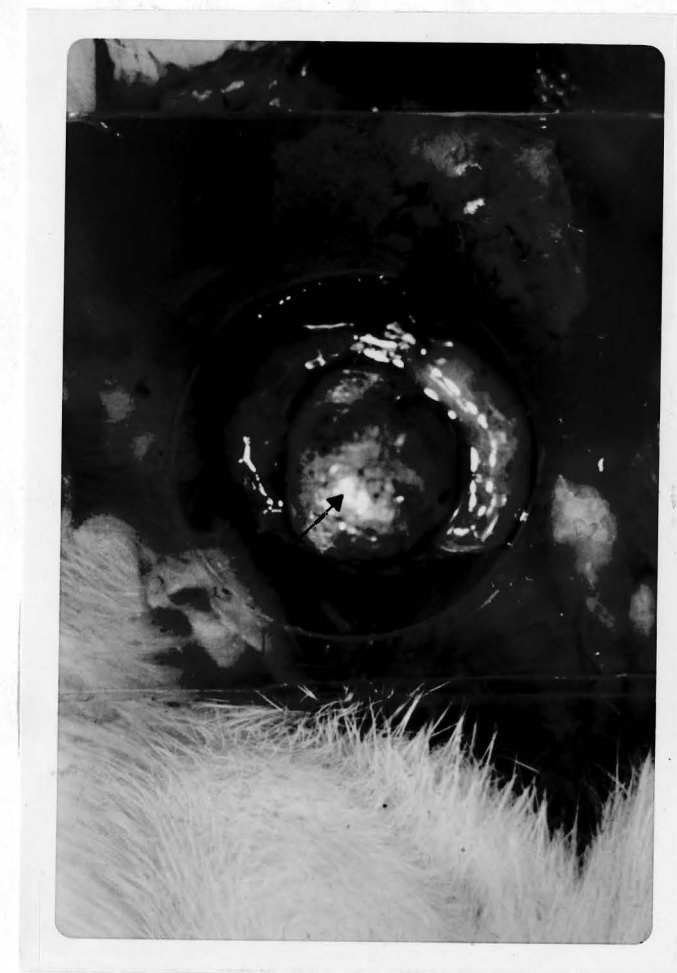


FIGURE 6

Figure 7 is the cross section of Figure 6
(fore-stomach) showing extensive
edema and hyperemia in the mucosa and
submucosa (stained with H & E; x 100).

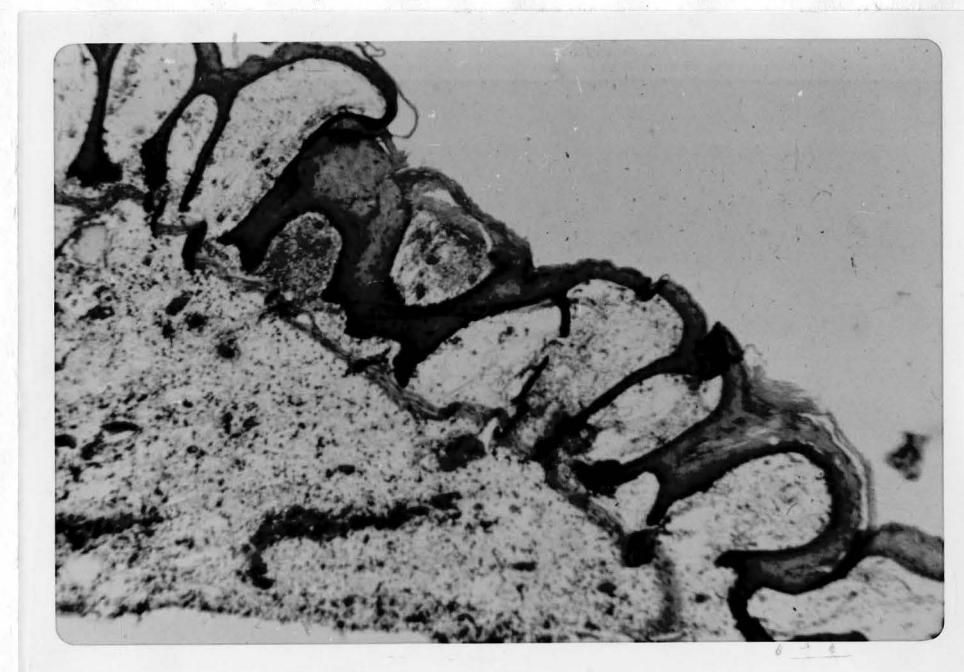


FIGURE 7

Figure 8 is a light micrograph of stomach of rat fed with water for 3 months (control). Note the intact mucosal cells and glands (stained with H & E; x 360).

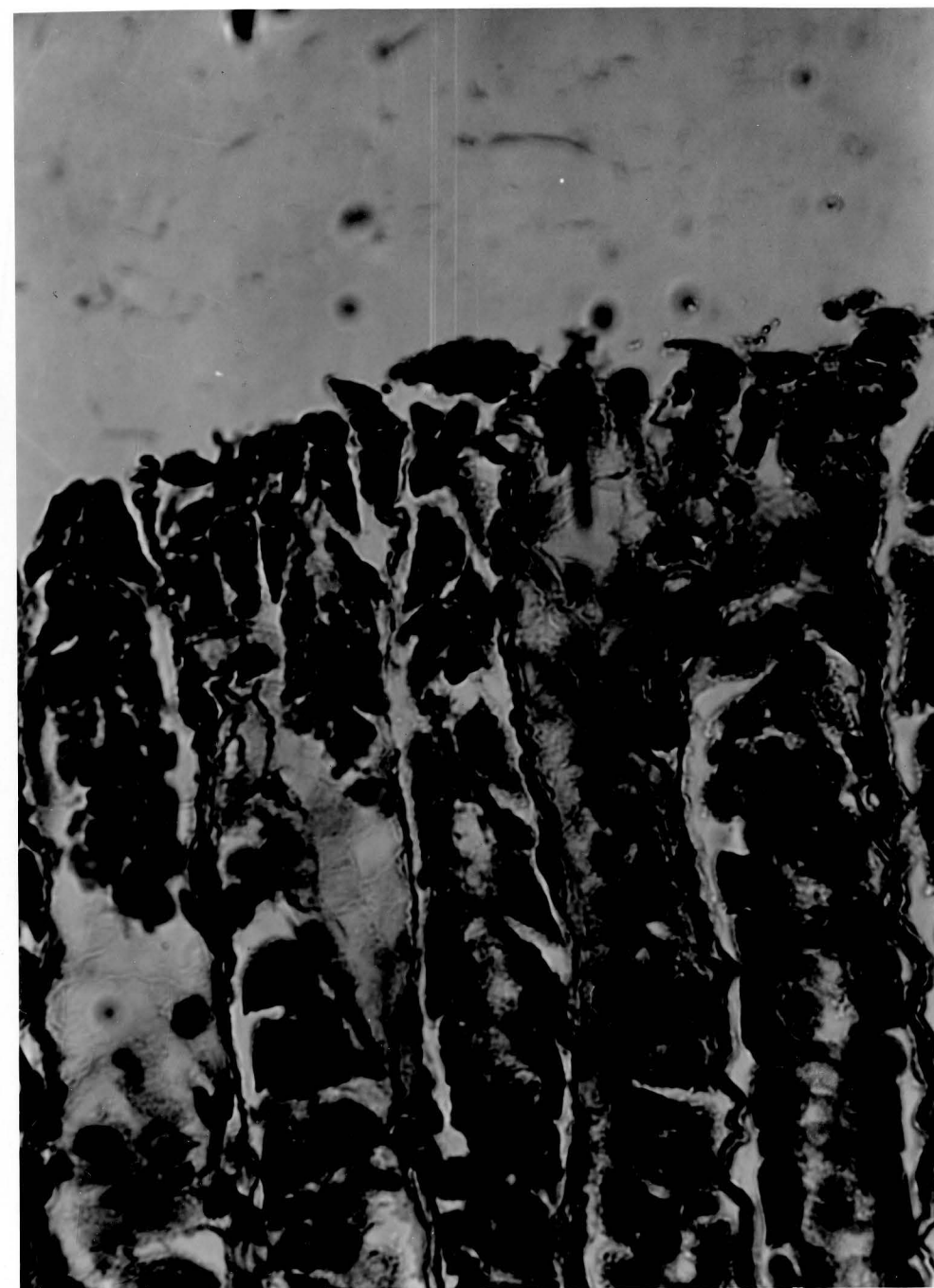


FIGURE 8

Figure 8a is a light micrograph of stomach of rat fed with 5% ethanol for 3 months (control). Note the intact mucosal cells and glands (stained with H & E; x 360).

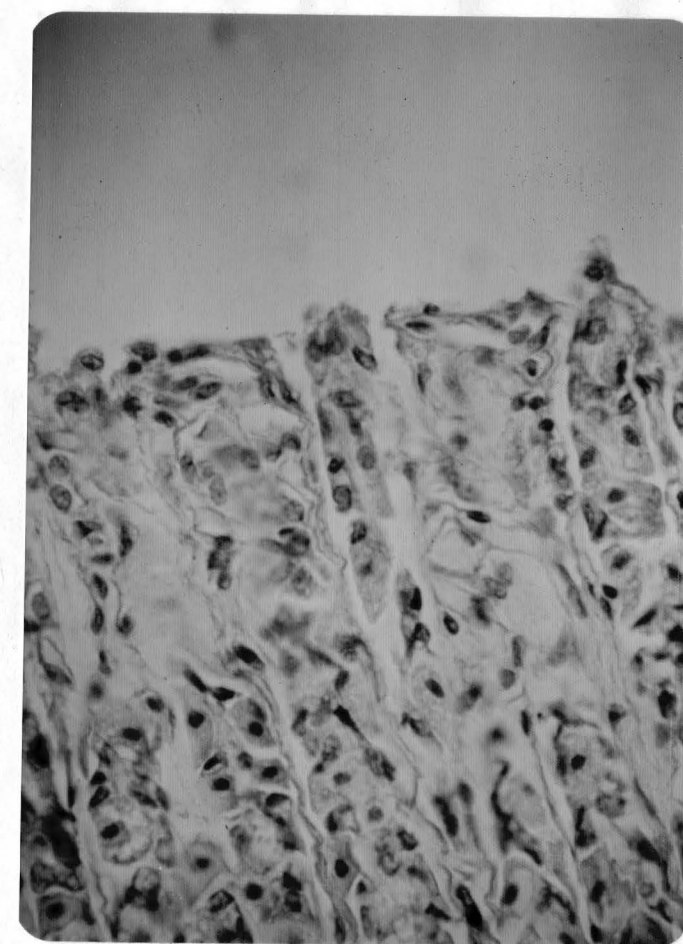


FIGURE 8a

Figure 9 is a light micrograph of rat stomach fed with red hot pepper extract for 3 months. The blood vessels are not only dilated but in some areas they are exposed to the lumen by the loss of the lining epithelium (stained with H & E; x 360).

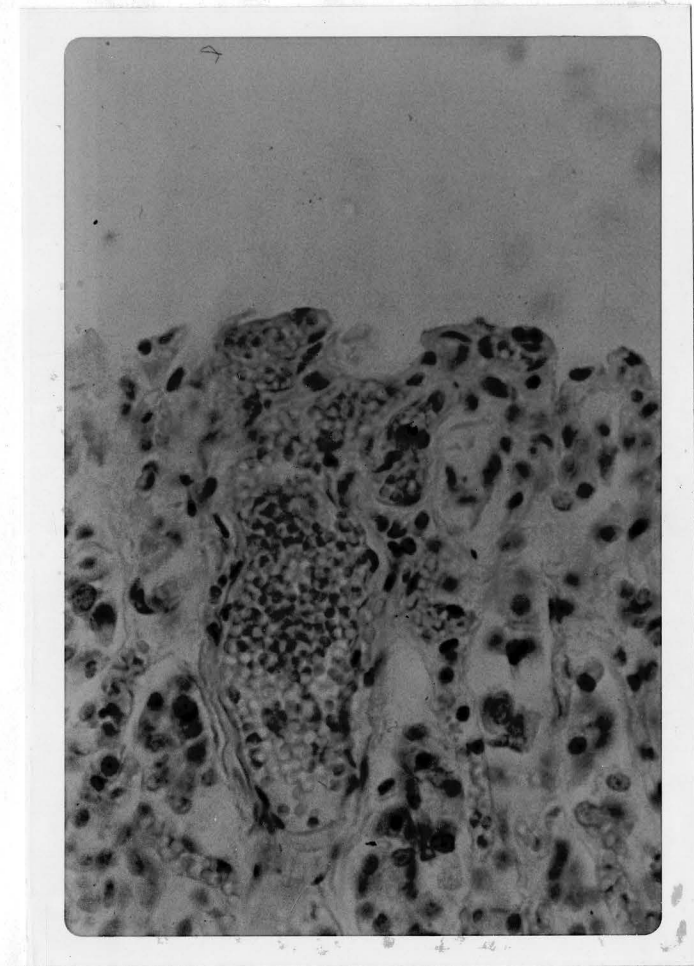


FIGURE 9

Figure 9a: The rat was fed with capsaicine for 3 months. There is acute inflammation and hyperemia. There is a loss of superficial gastric epithelium. The gastric content was bloodstained. Some capillaries were completely disrupted and there was associated loss of red blood cells into the adjacent interstitial areas and into the lumen (stained with H & E; x 360).

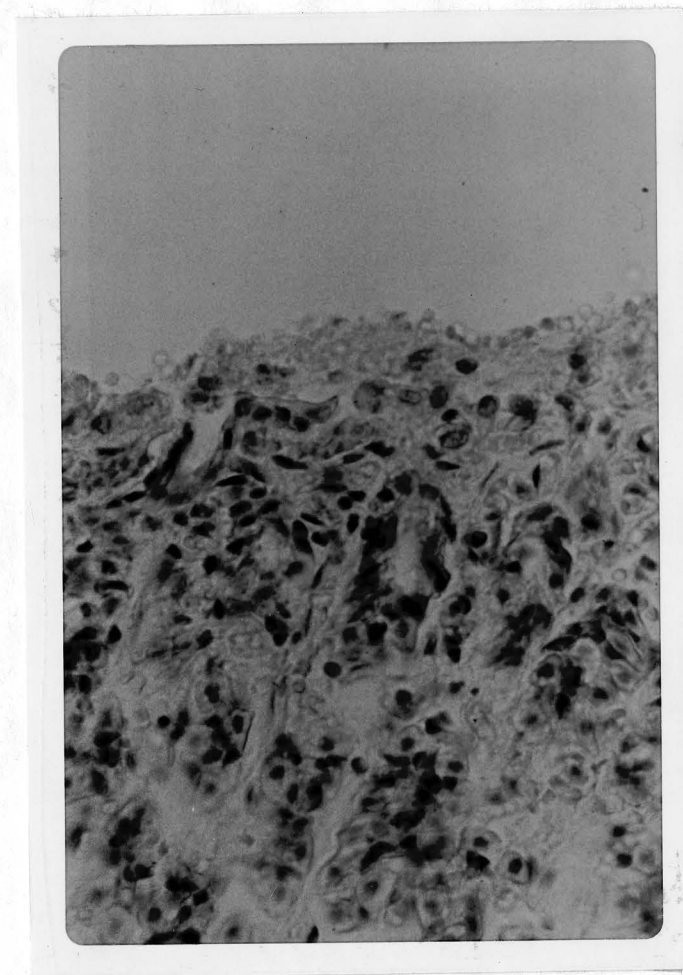


FIGURE 9a

Figure 10 shows rat stomach fed with water (control), for 6 months. The superficial gastric epithelium is intact and the glands orderly (stained with H & E; x 360).

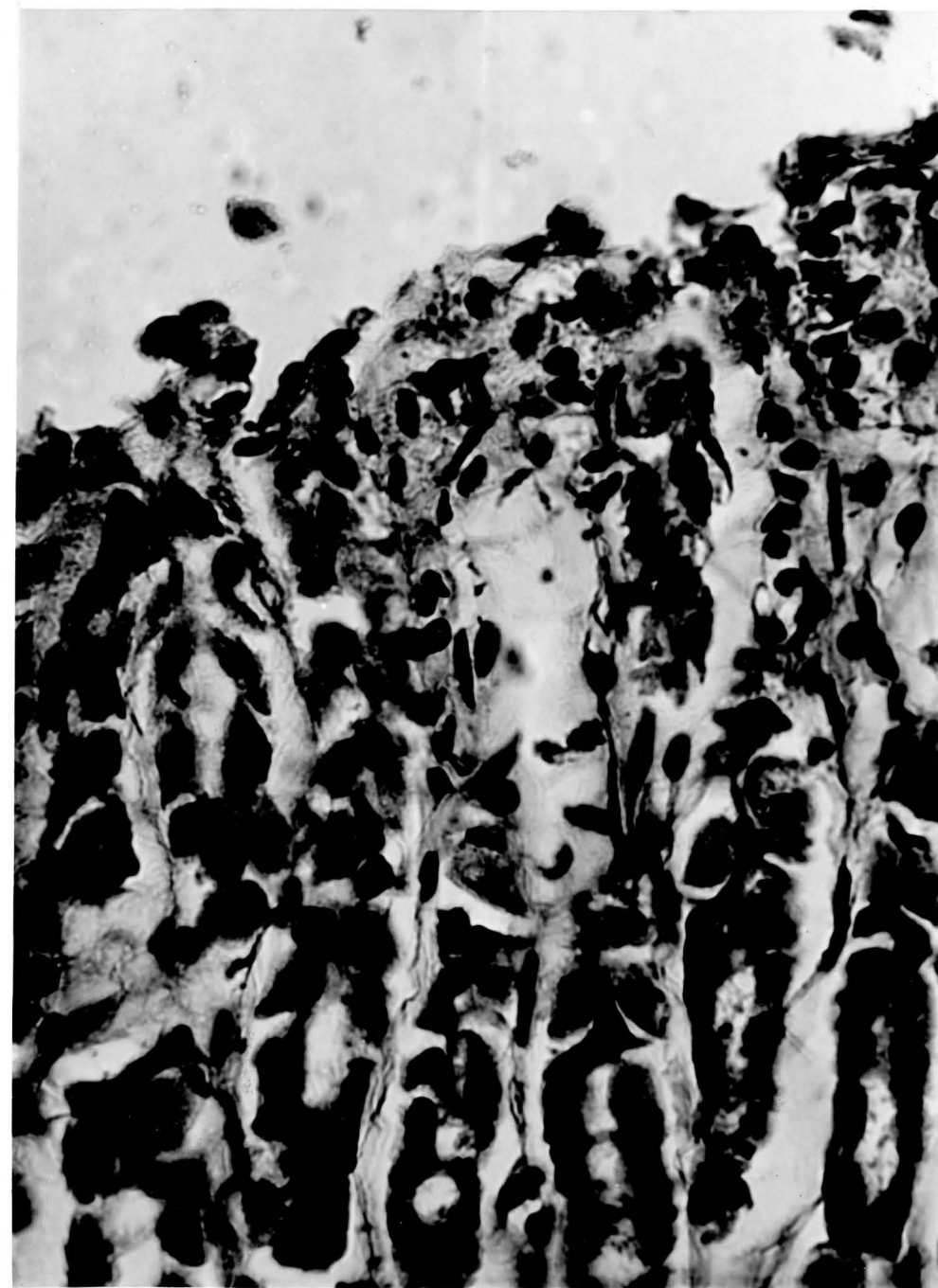


FIGURE 10

Figure 10a shows rat stomach fed with 5% ethanol (control), for 6 months. The superficial gastric epithelium is intact and the glands orderly (stained with H & E; x 360).

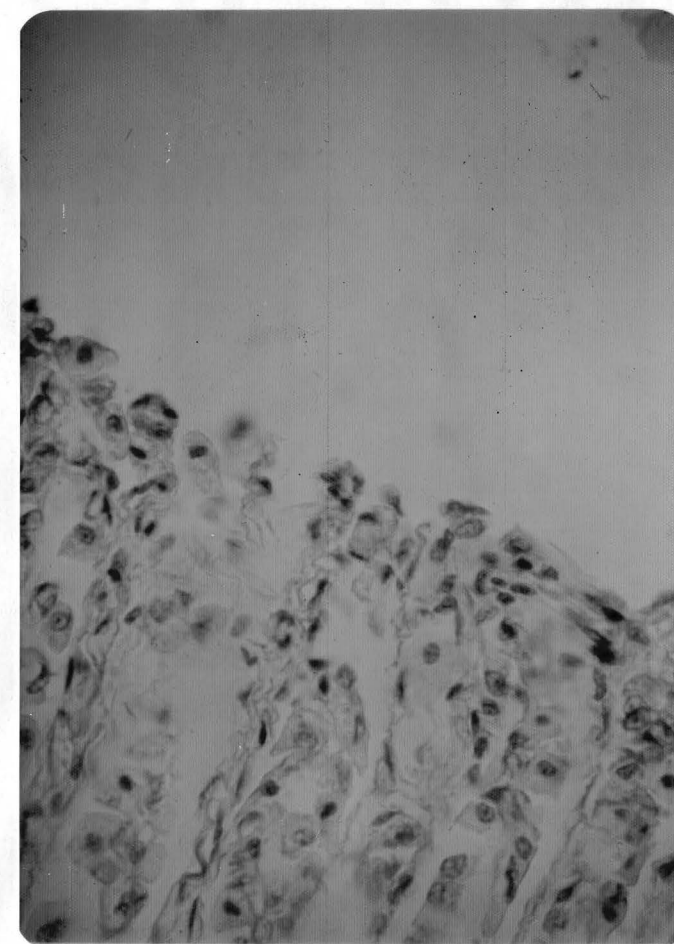


FIGURE 10a

Figure 11 is the rat gastric mucosa fed
with red pepper extract for
6 months.

Figure 11a is the rat gastric mucosa fed
with capsaicine solution for
6 months.

In both cases, there is a dilation or a rupture
of the subendothelial blood vessels and loss of
superficial epithelium in the gastric mucosa. The
gastric content was bloodstained (both stained
with H & E; x 360).

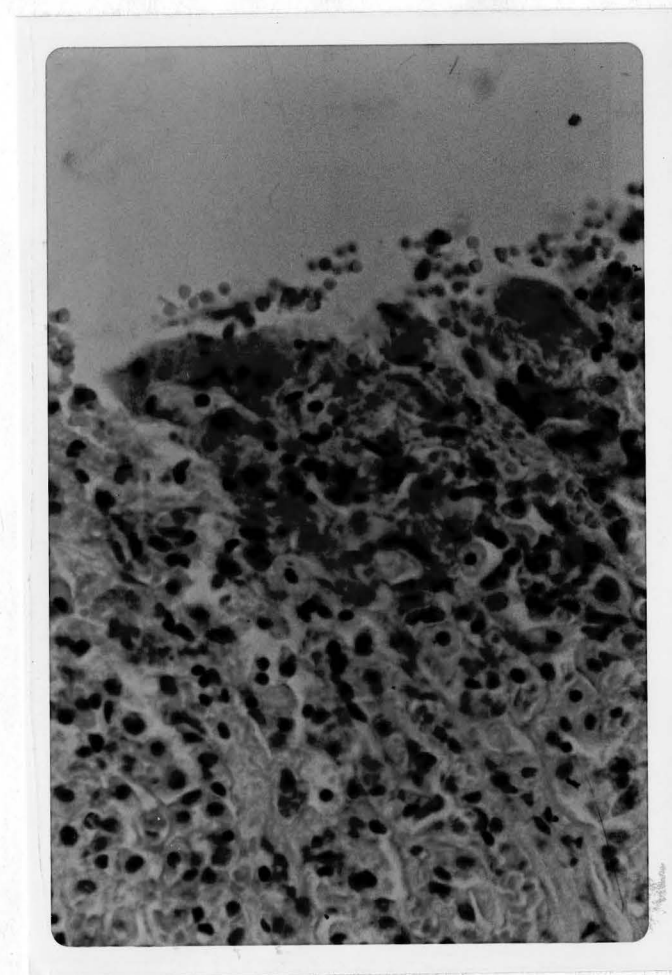


FIGURE 11



FIGURE 11a

Figure 12 shows rat stomach fed with
water for 9 months (control).
The gastric mucosa is intact
with normal glands and foveolae
(stained with H & E; x 360).

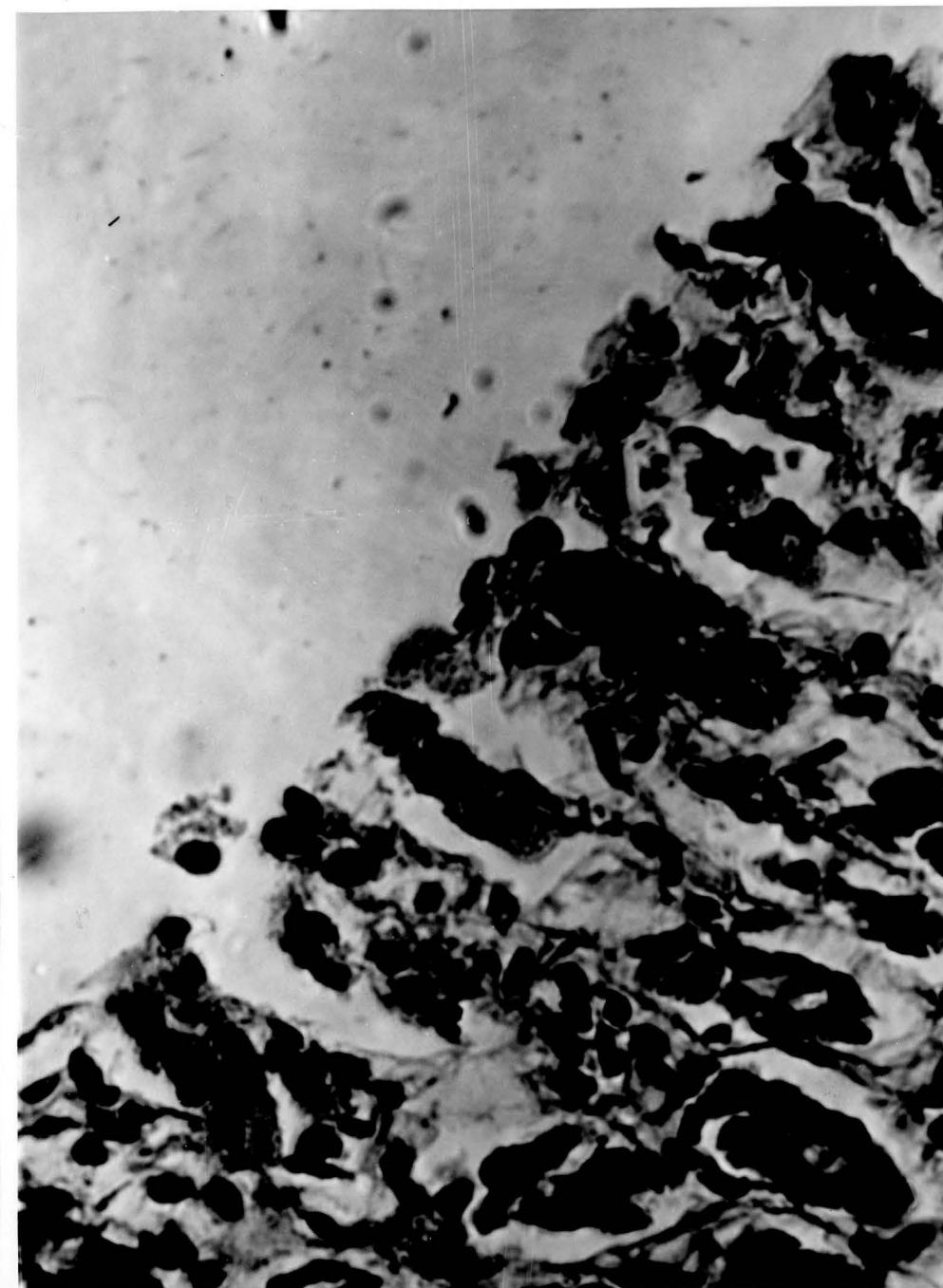


FIGURE 12

Figure 12a shows rat stomach fed with
5% ethanol for 9 months (control).
The gastric mucosa is intact with
normal glands and foveolae (stained
with H & E; x 360).

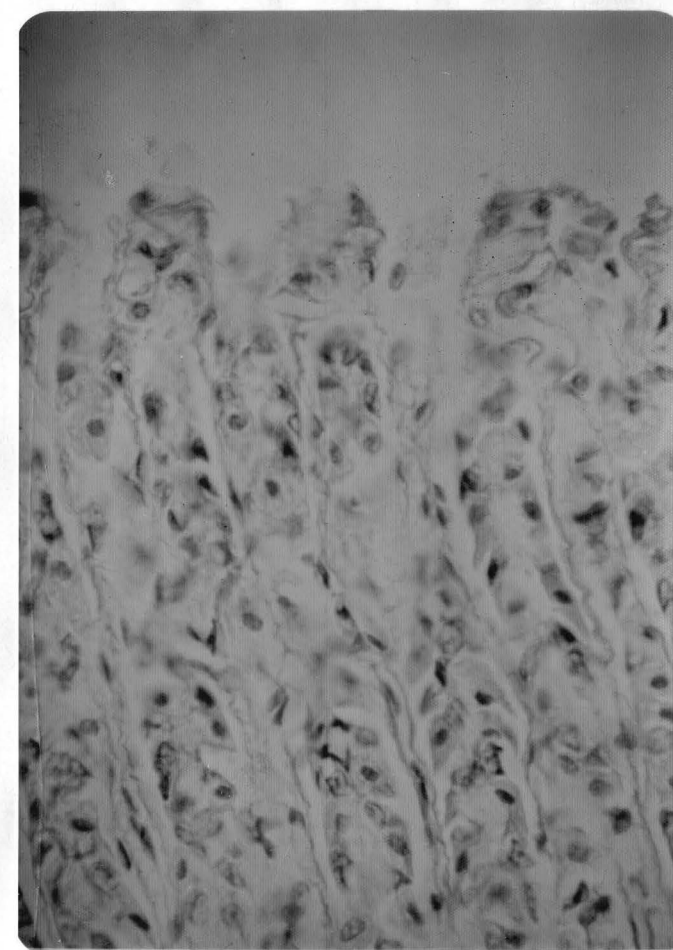


FIGURE 12a

Figure 13 is the rat gastric mucosa fed with red pepper extract for 9 months (stained with H & E; x 360).

Figure 13a is the rat gastric mucosa fed with capsaicine solution for 9 months (stained with H & E; x 360).

Both show acute inflammation, hyperemia, glandular degeneration and intertubular edema. Some capillaries have ruptured and some have been exposed to the lumen by the loss of the lining epithelium. Some of the glands have dilated as indicated by the arrows.

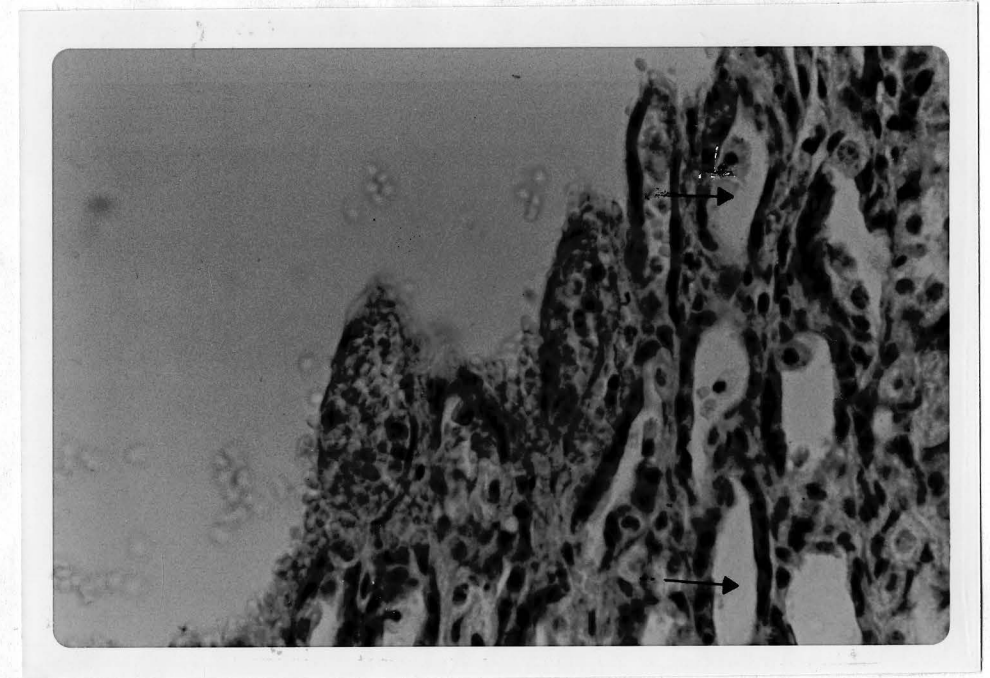


FIGURE 13

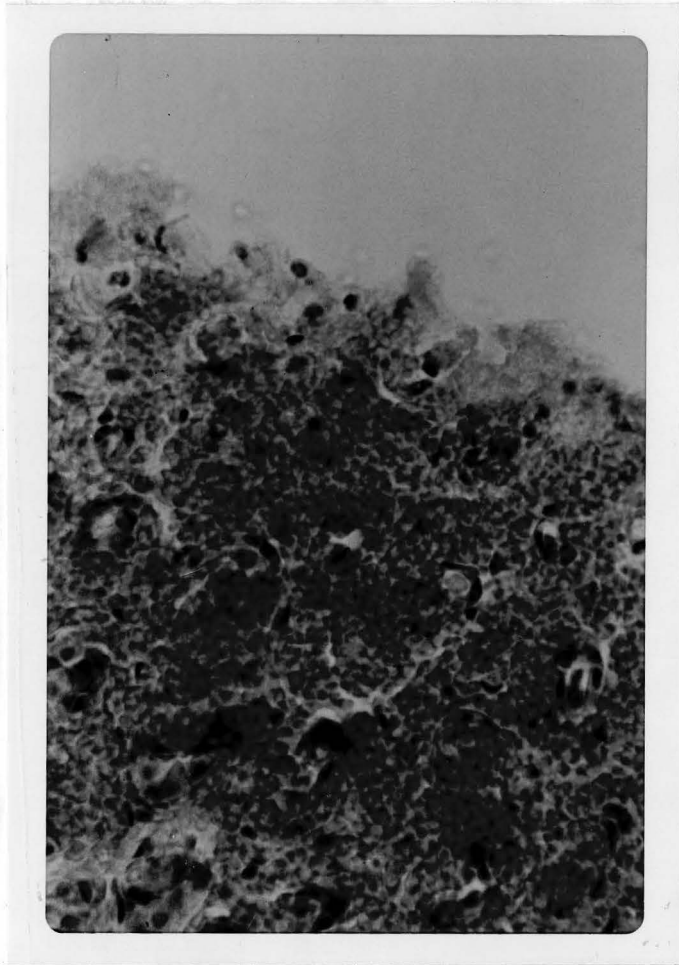


FIGURE 13a

Figure 13b: The occurrence of morphological changes in long term rats intubated with Capsicum or capsaicine and their appropriate water or ethyl alcohol controls for 3, 6 or 9 months.

LONG TERM EFFECTS OF CAPSICUM OR CAPSAICINE ON RAT STOMACH

DURATION	NO. OF RATS	BLOOD IN GAS. LUMEN	DAMAGE TO MUCOSA	DAMAGE TO SUBMUCOSA	DILATION OF GLANDS
3 MONTHS	EXPERIMENTAL	I2	I2	I2	-
	CONTROL	8	-	-	-
6 MONTHS	EXPERIMENTAL	I2	I2	I2	5
	CONTROL	8	-	-	-
9 MONTHS	EXPERIMENTAL	II	II	II	II
	CONTROL	7	-	-	-

FIGURE 13b

Figure 14 is a rectangular Luxite box used for the operation. The gastric mucosa of the rat is exposed.

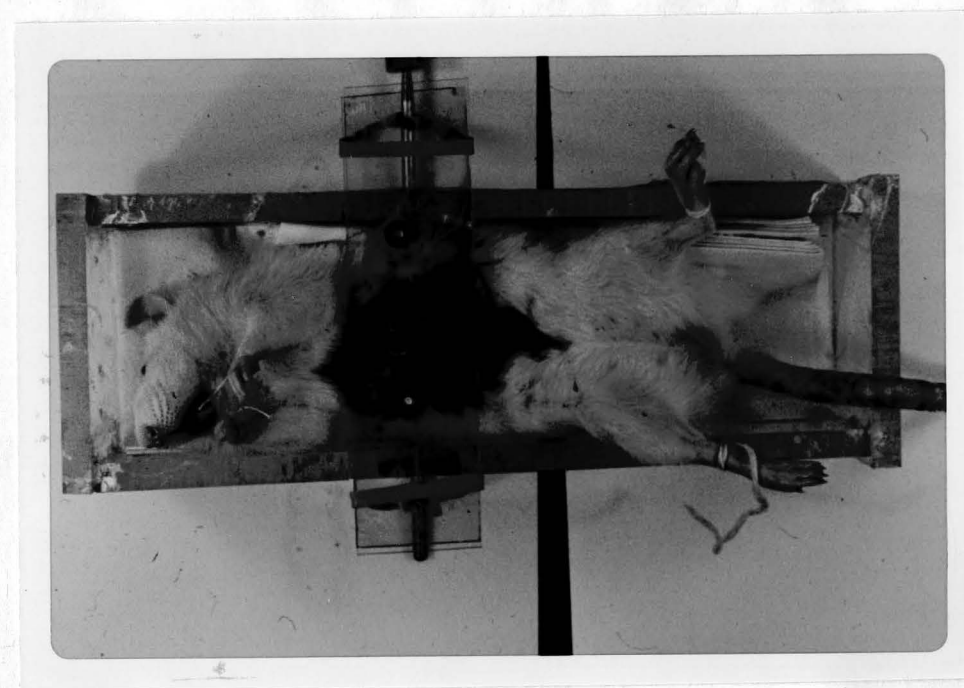


FIGURE 14

Figure 15 shows the rat-stomach irrigated with 5% alcohol for 30 minutes (control). A number of gastric glands as well as pits and the free luminal surface are shown. The epithelium is intact (stained with H & E; x 360).

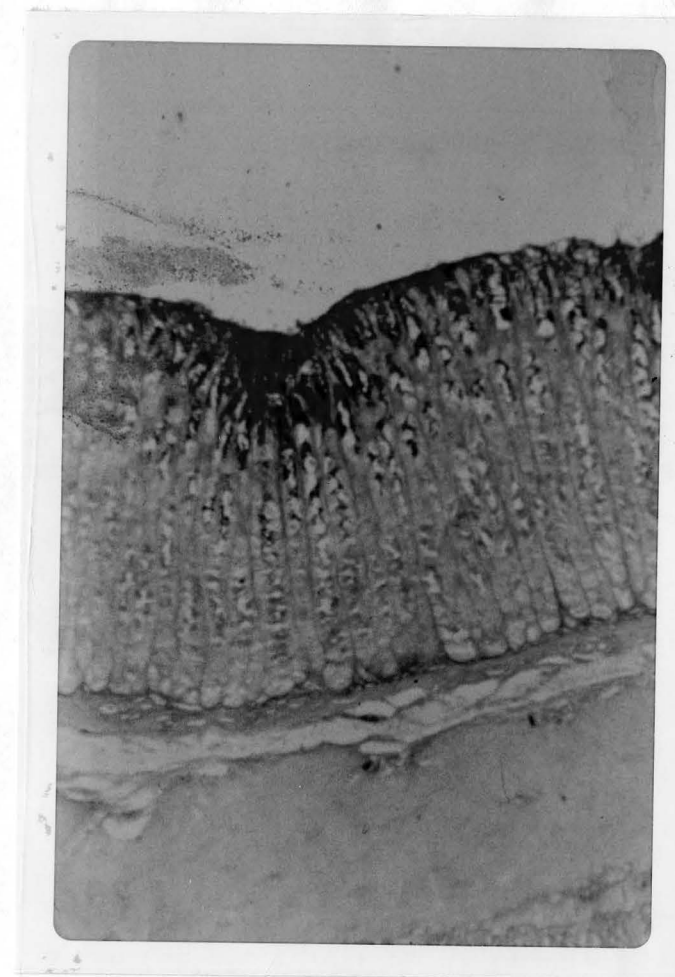


FIGURE 15

Figure 16 is rat stomach exposed to capsaicine for 30 minutes and stained with Alcian blue and PAS (x100). Some areas of the mucosa are necrotic and there is a sloughing of superficial cells.

Figure 16a is the rat gastric mucosa exposed to capsaicine for 30 minutes and stained with H & E (x 360). The capillaries are dilated and congested with red blood cells. Some of the capillaries are exposed and some have ruptured.



FIGURE 16

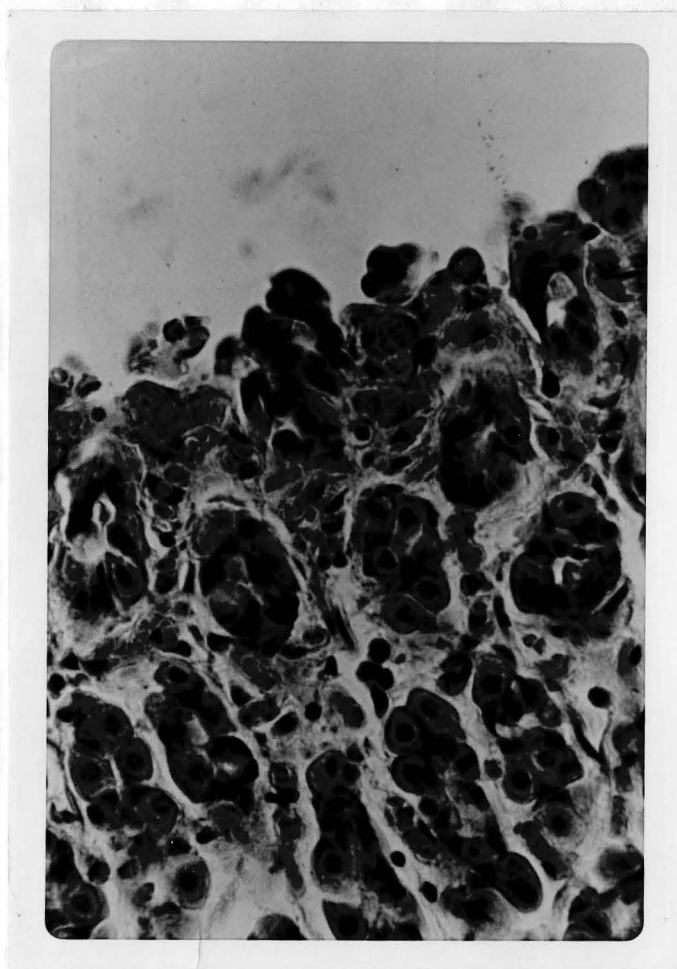


FIGURE 16a

Figure 17 is rat gastric mucosa exposed to 5% ethanol for 60 minutes. The glands and the epithelium are normal (stained with H & E; x 360).

Figure 17a is the rat gastric mucosa (1 u thick) exposed to 5% ethanol for 60 minutes. Capillaries, 1 cell thick in diameter are prominent in the lamina propria. Cell apices are smooth and rounded (stained with Toluidine blue; x 780).



FIGURE 17

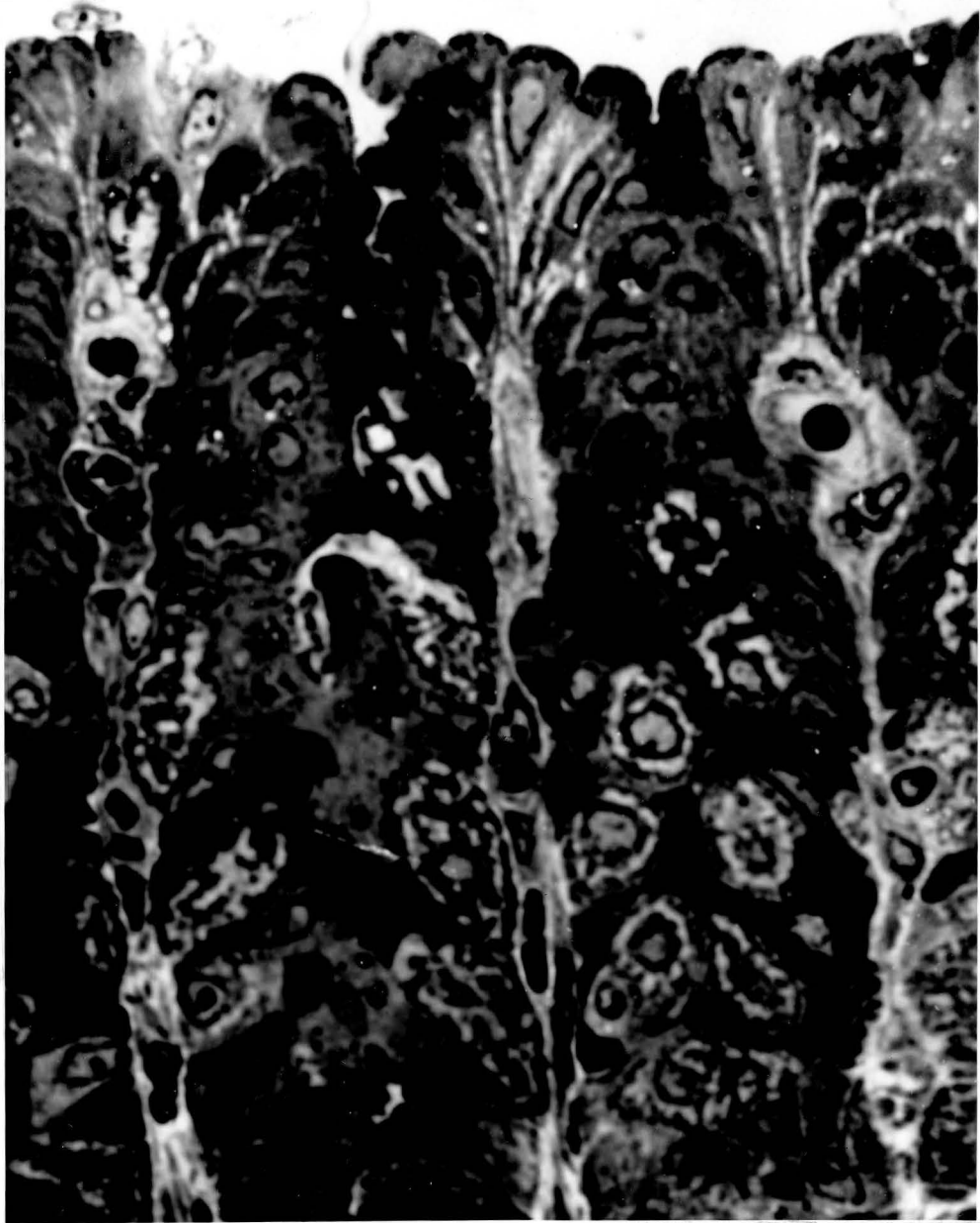


FIGURE 17a

Figures 18 and 18a are rat stomach exposed to capsaicine for 60 minutes. The surface epithelial cells have been severely damaged and their destruction has exposed dilated capillaries within whose lumen the red cells have hemolyzed. Underlying gland cells and deeper blood vessels show no damage.

Figure 18 is 7u thick and stained with H & E (x360).

Figure 18a is 1u thick and stained with Toluidine blue (x 780).

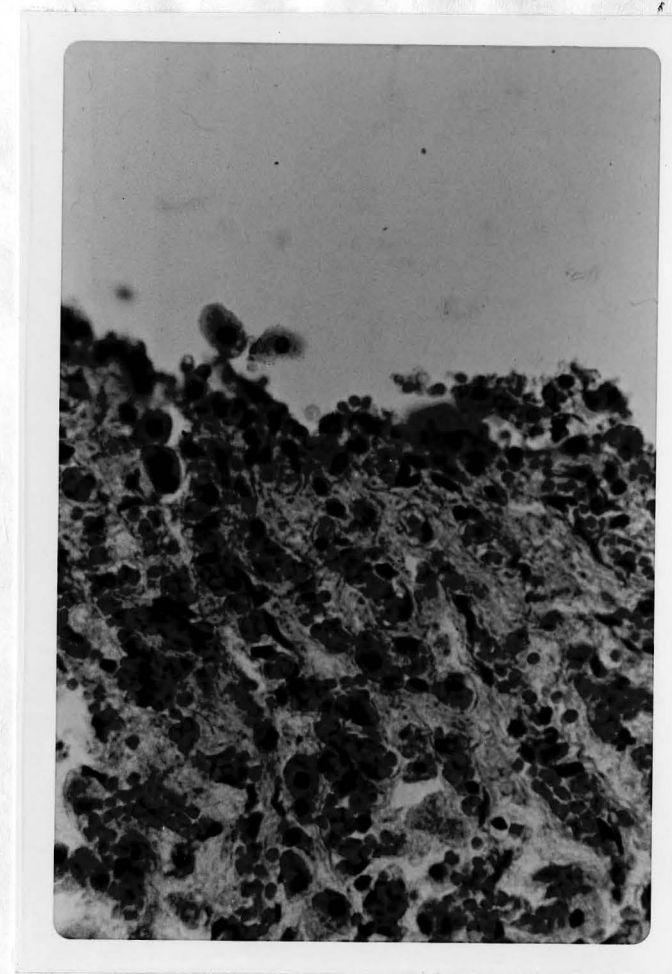


FIGURE 18

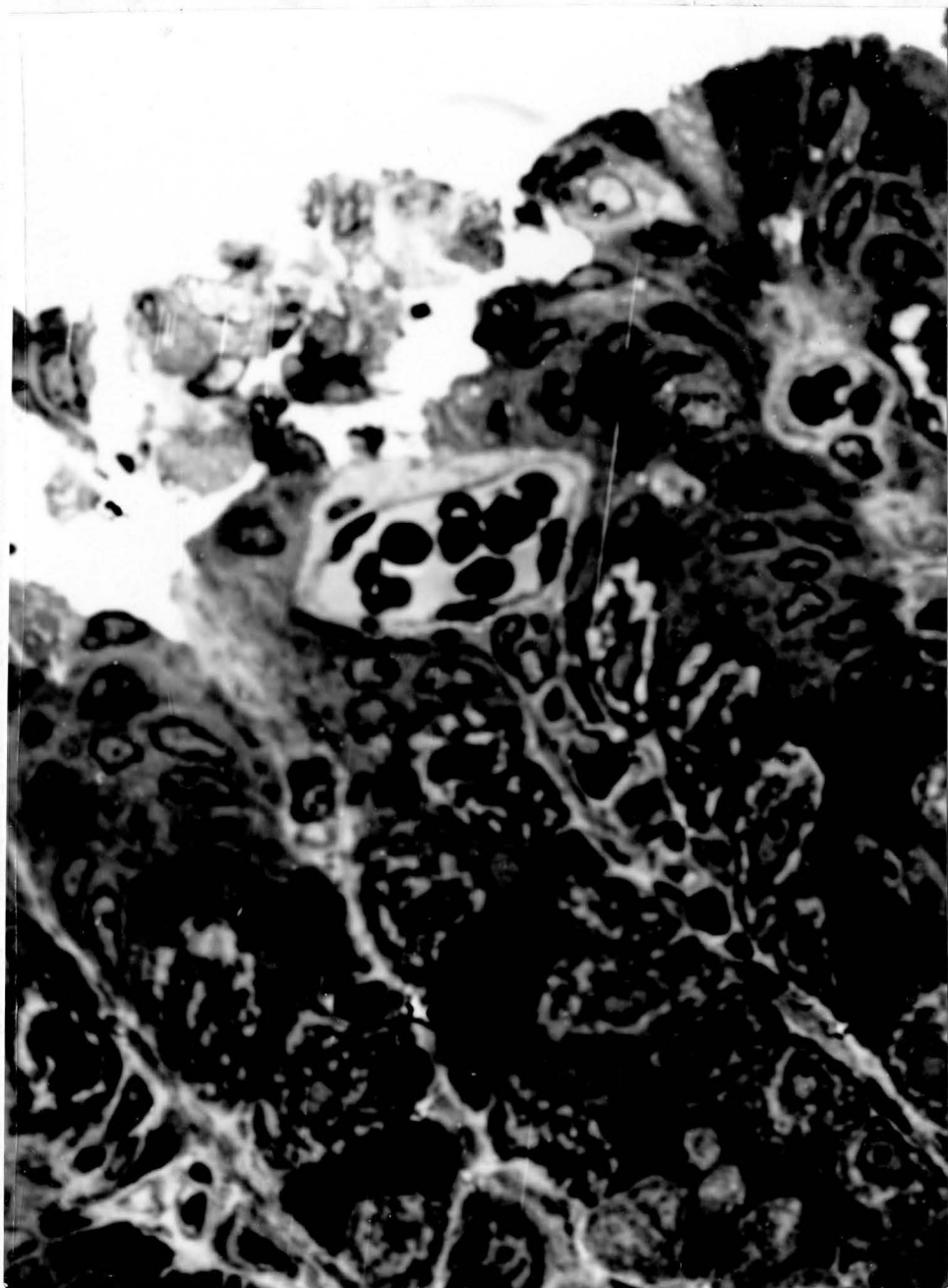


FIGURE 18a

Figure 19 is the rat stomach exposed to 5% alcohol for 90 minutes (control). The surface mucous cells are normal (stained with H & E; x 325).



FIGURE 19

Figure 20 is the rat gastric mucosa exposed to capsaicine for 90 minutes. It shows part of an erosion in the gastric mucosa with some dilation and some exposure of blood vessels to the gastric lumen (stained with H & E; x 325).

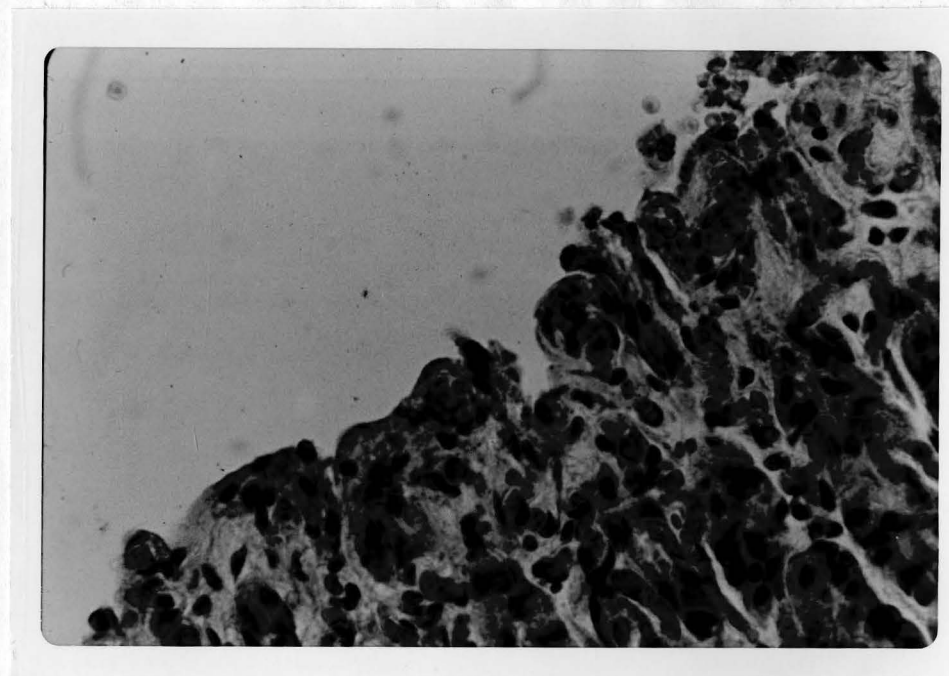


FIGURE 20

Figure 21 is scanning electron micrograph of normal surface of the fundus of the rat stomach. Note the convex surfaces of mucous epithelial cells and mucosal surface convolutions with gastric pits (x 2,000).

Figure 22 shows normal surface of the fundus of the rat stomach (x 8,750). Note the presence of microvilli (arrows).

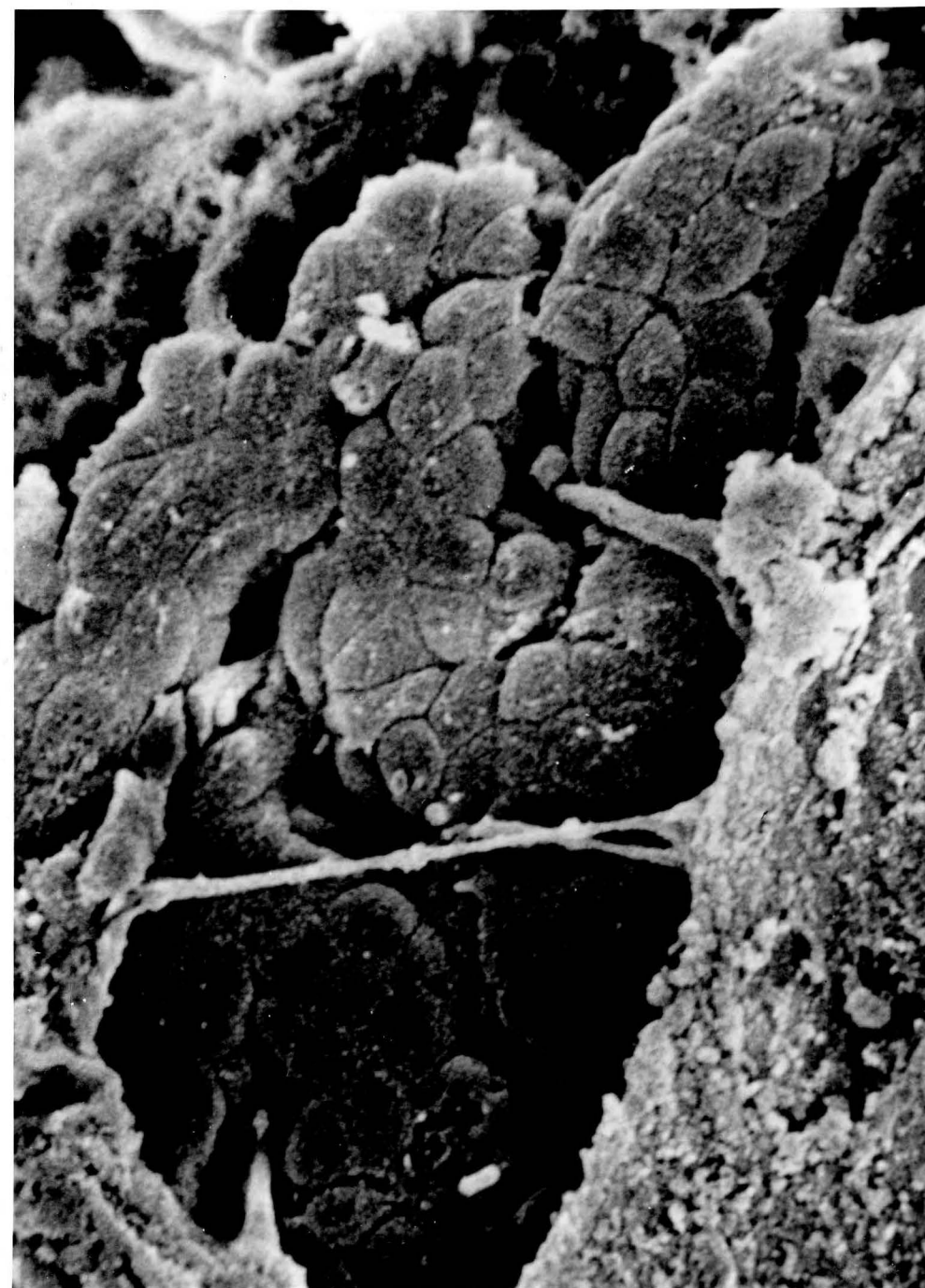


FIGURE 21



FIGURE 22

Figure 23 is scanning electron micrograph of rat gastric luminal surface exposed to 5% ethanol for 30 minutes, as control. It shows epithelial cells covered for the most part by a sheet of mucous. At the bottom left and upper right are areas of interrupted mucous showing the underlying cells. Arrows indicate foveolae (x 500).

Figure 24 shows some of the normal characteristics of the surface mucous cells: continuous sheet of gastric epithelial cells with microvilli (x 14,000).

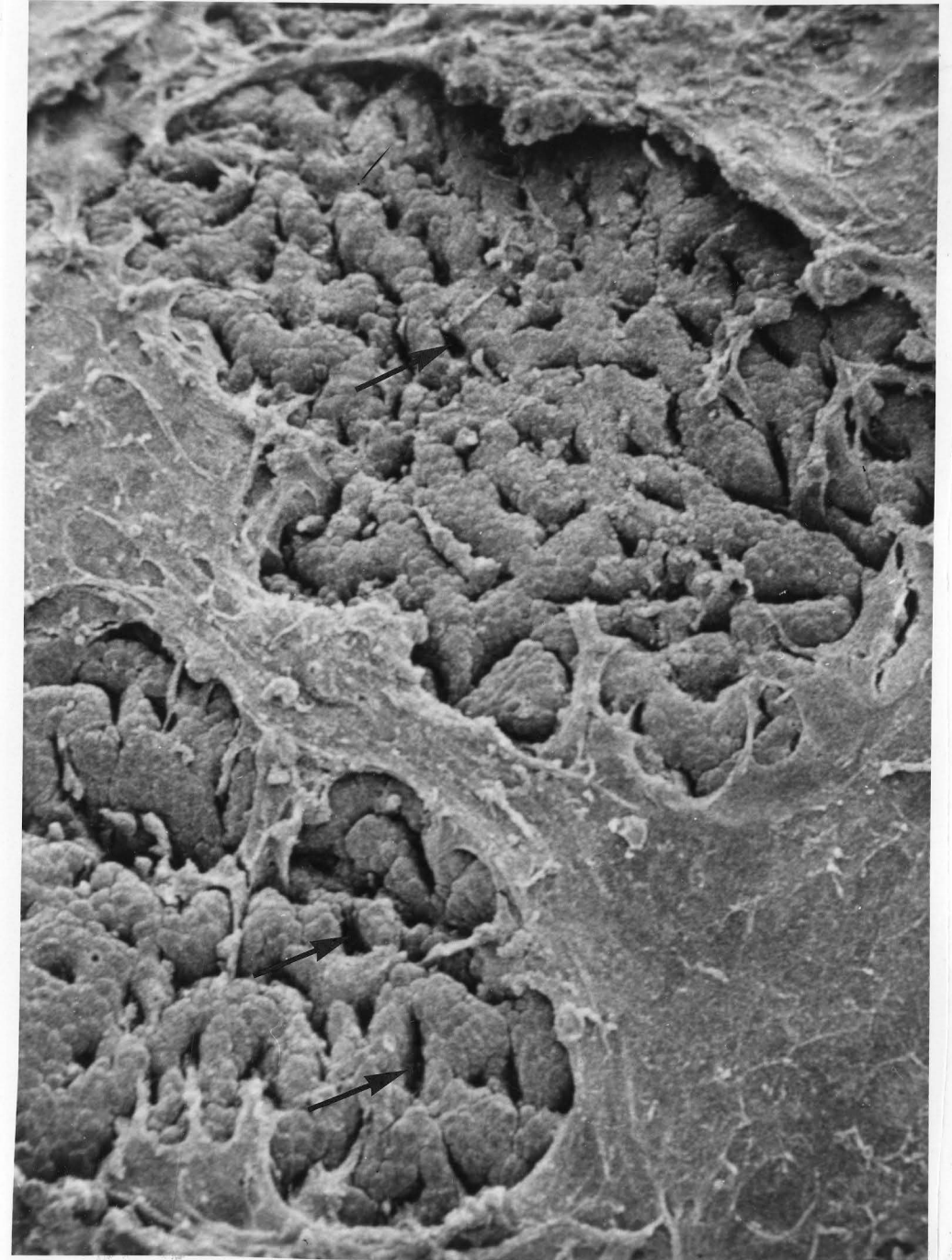


FIGURE 23

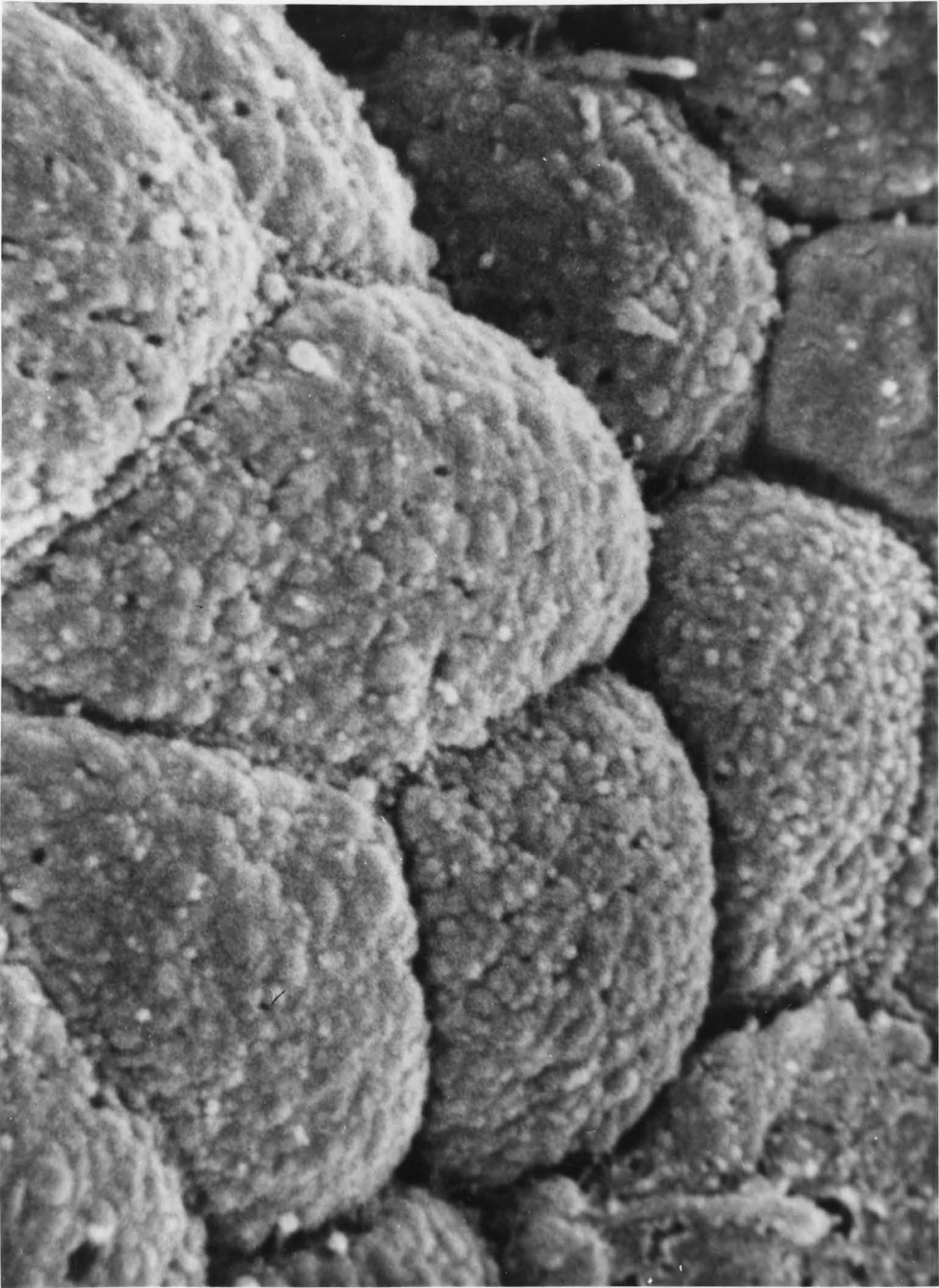


FIGURE 24

Figure 25 is scanning electron micrograph of the luminal surface of rat gastric mucosa exposed to capsaicine for 30 minutes. Note localized mucosal surface damage (erosion) caused by capsaicine at the lower left. Note also the morphologically normal cells adjacent to the erosion (x 1,400).

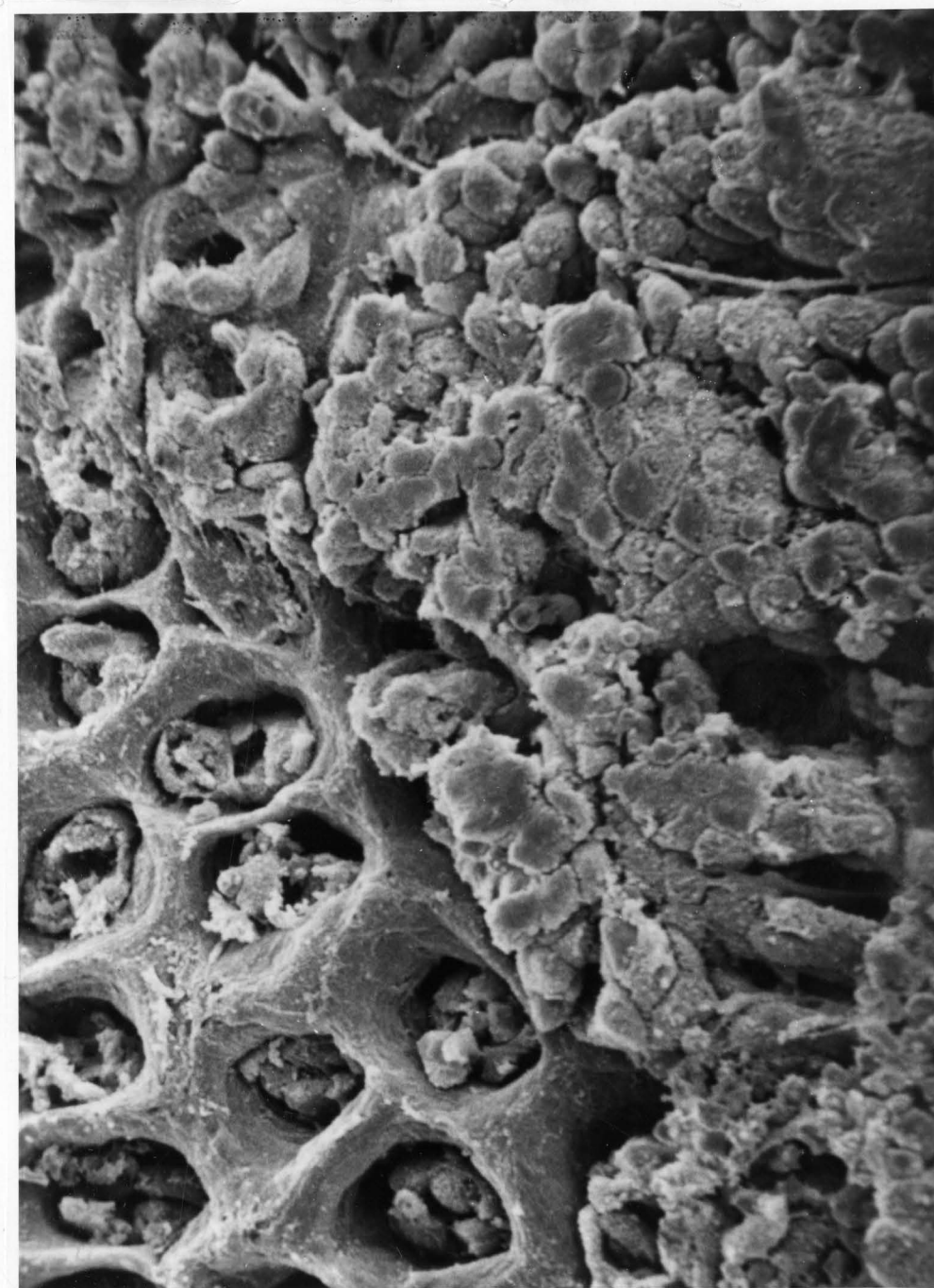


FIGURE 25

Figure 26 is scanning electron micrograph of the luminal surface of rat gastric mucosa exposed to capsaicine for 30 minutes. It shows the abrupt border of the lesion in which can be seen a few intact cells on the left hand side. This type of lesion is the primary source of blood loss (x 4,500).

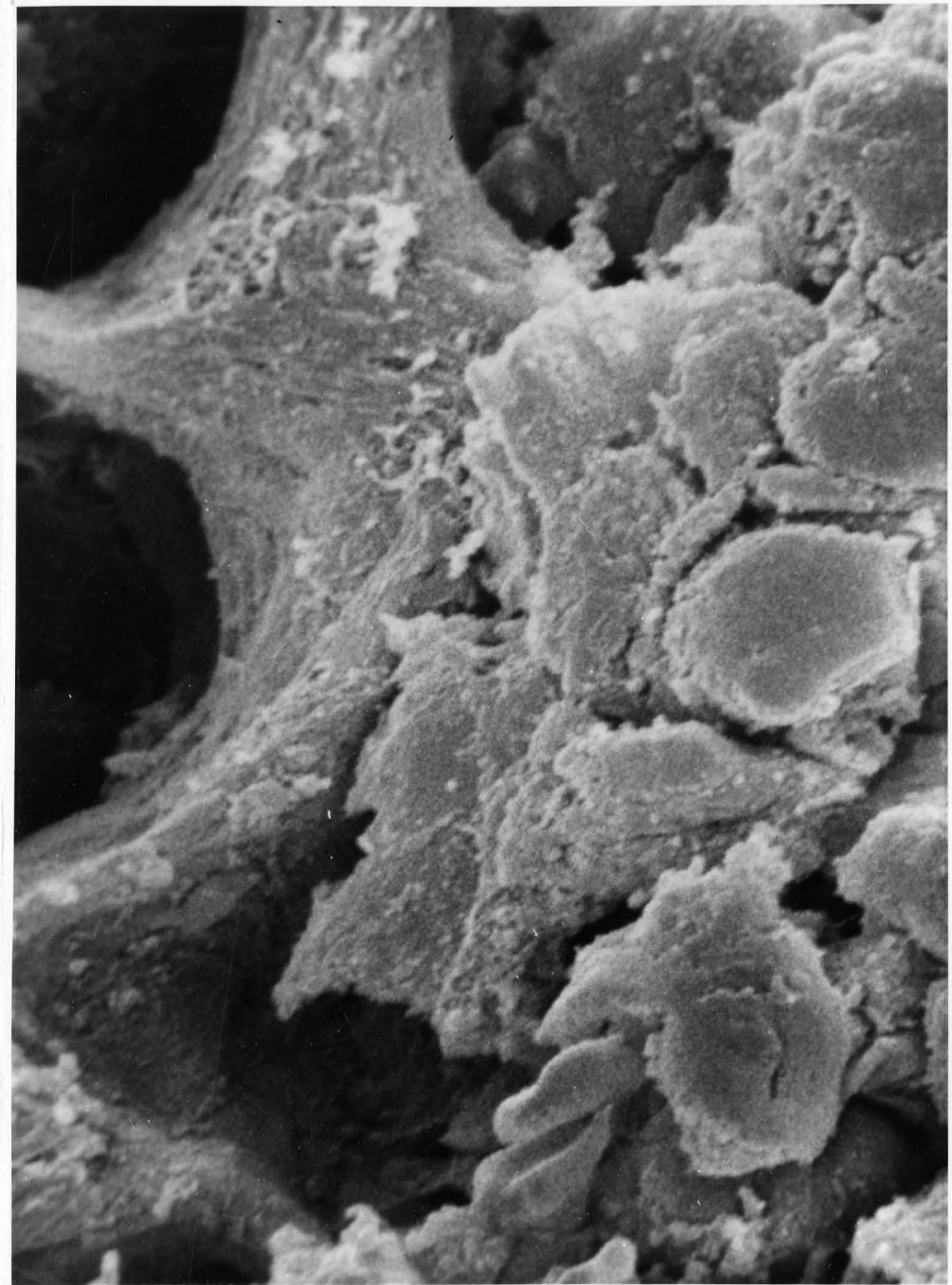


FIGURE 26

Figure 27 is scanning electron micrograph of the luminal surface of rat gastric mucosa exposed to 5% ethanol for 60 minutes as control. The mucosal cells are normal. There are no erosions. There is a continuous sheet of gastric epithelial cells. Note the microvilli at higher magnification (x 1,830).

Figure 28 is the scanning electron micrograph of the same specimen shown in Figure 27 at higher magnification. Note the microvilli (x 14,000).

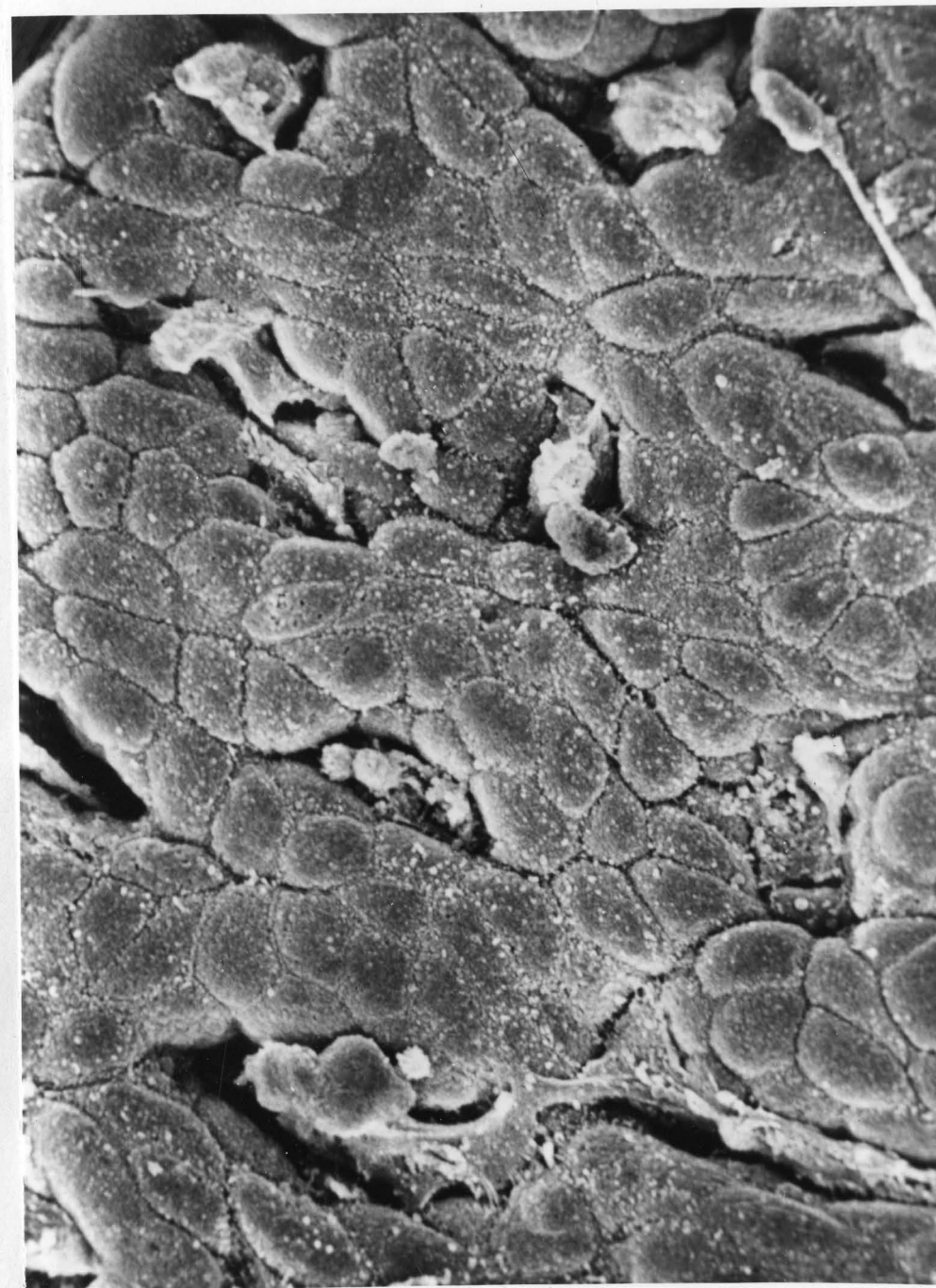


FIGURE 27



FIGURE 28

Figure 29 is scanning electron micrograph of the luminal surface of rat gastric mucosa exposed to capsaicine for 60 minutes. Note the residual framework of connective tissue with disrupted epithelial cells. There is a definite loss of cell contents. The arrow indicates free red blood cells (x 1,250).

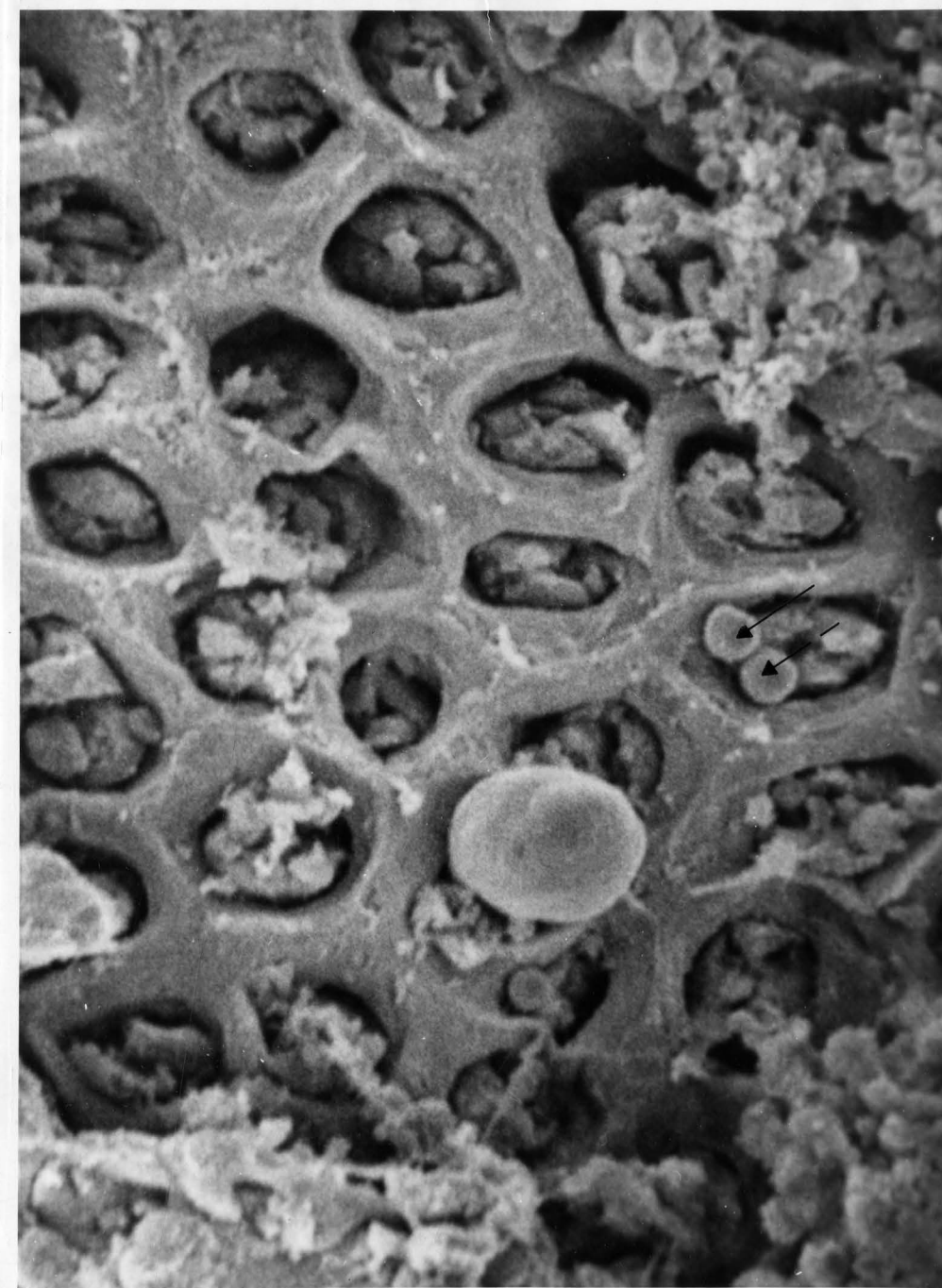


FIGURE 29

Figure 30 is scanning electron micrograph of rat gastric mucosa exposed to 5% ethanol (as control) for 90 minutes. The mucosal cells are normal (x 1,120).

Figure 31 is scanning electron micrograph of the same specimen shown in Figure 30 at higher magnification. Note the continuous sheet of gastric epithelial cells with microvilli (x 11,200).

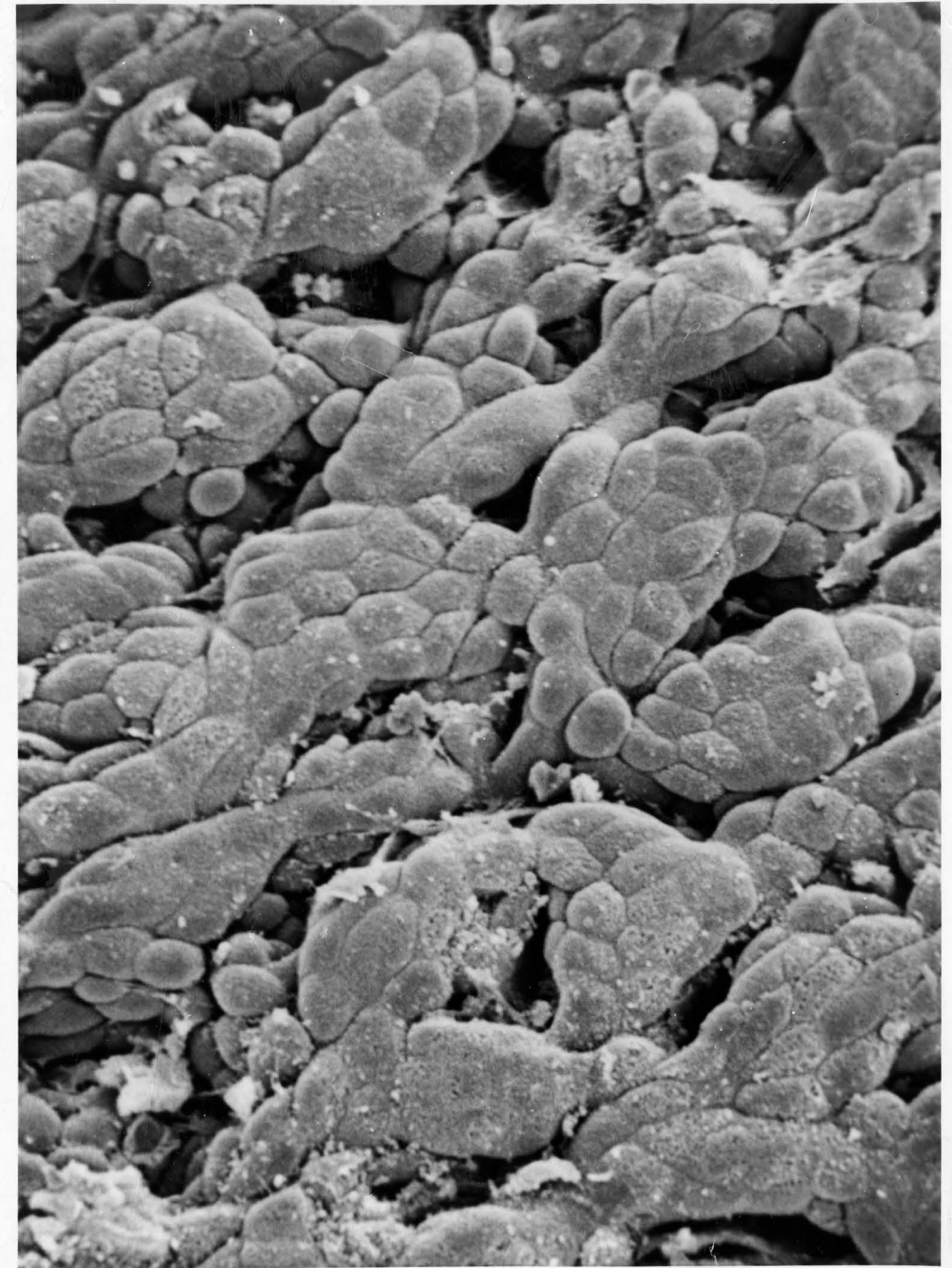


FIGURE 30

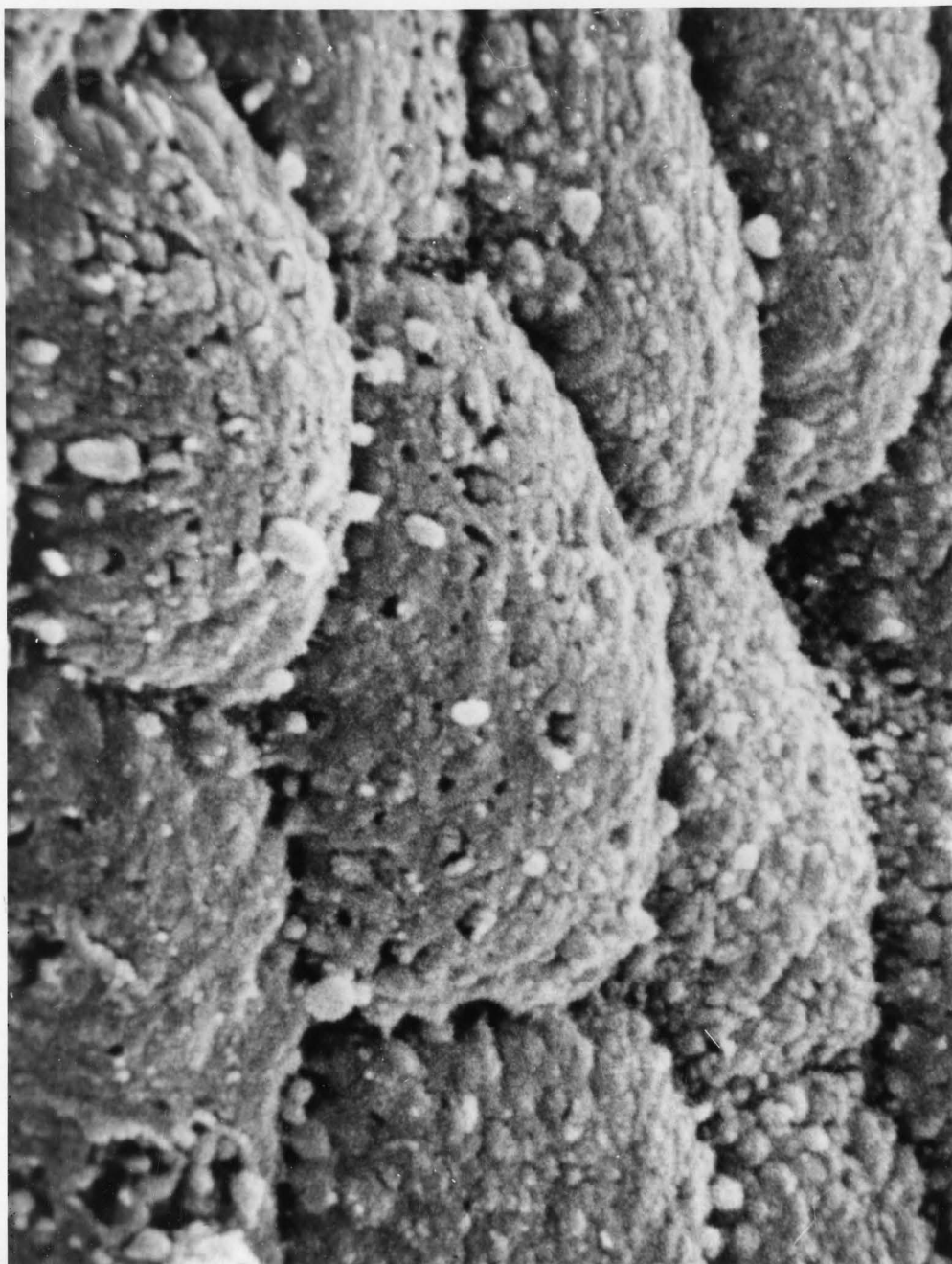


FIGURE 31

Figure 32 is the scanning electron micrograph of the luminal surface of rat gastric mucosa exposed to capsaicine for 90 minutes. There is an extensive disruption of gastric epithelial cells in some areas and in some, complete loss of surface epithelia and glands (x 1,225).

Figure 33 is scanning electron micrograph of the same specimen shown in Figure 32 at higher magnification. The epithelial cells have been sloughed off leaving behind a skeletal framework of connective tissue (x 5,225).

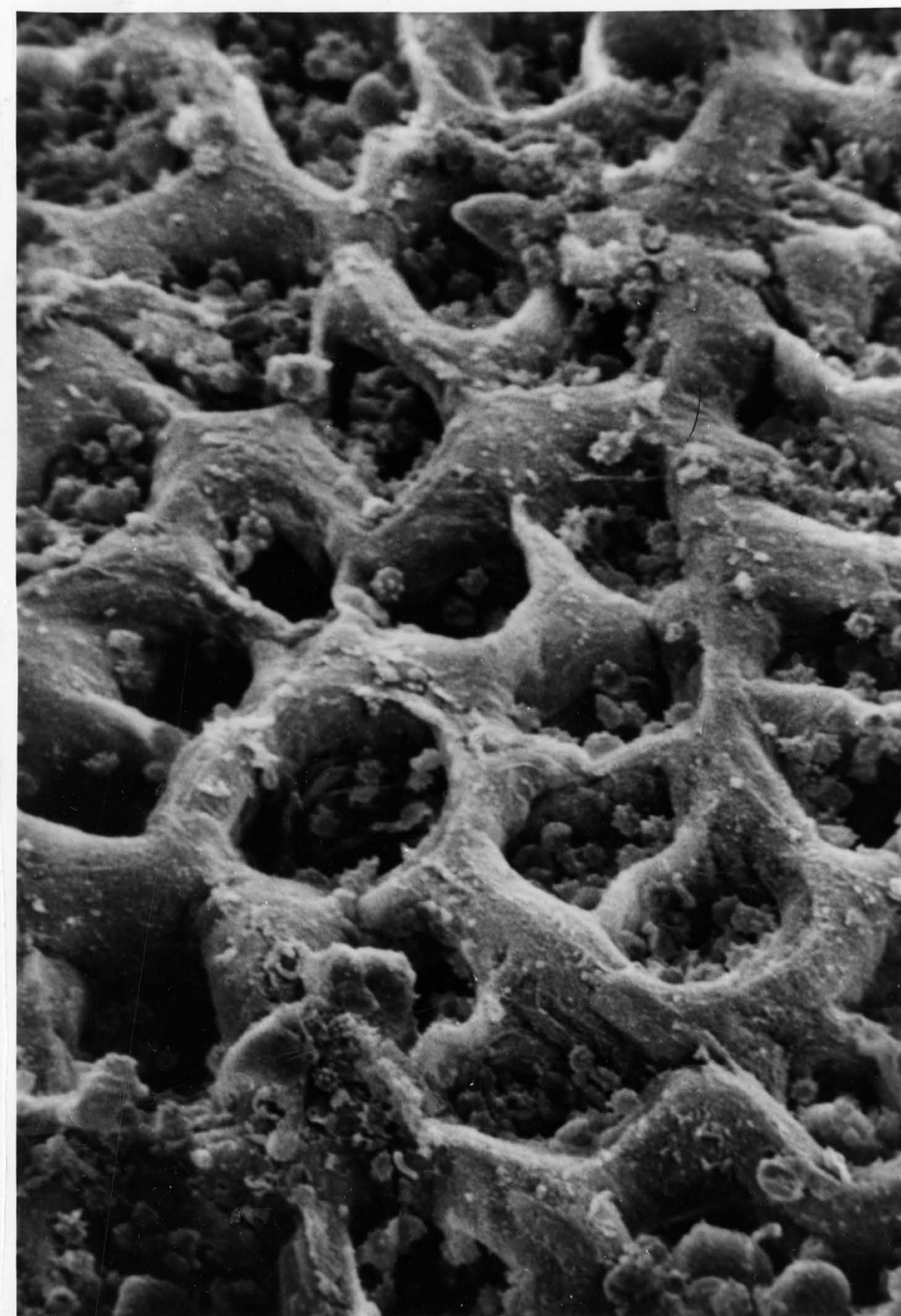


FIGURE 32

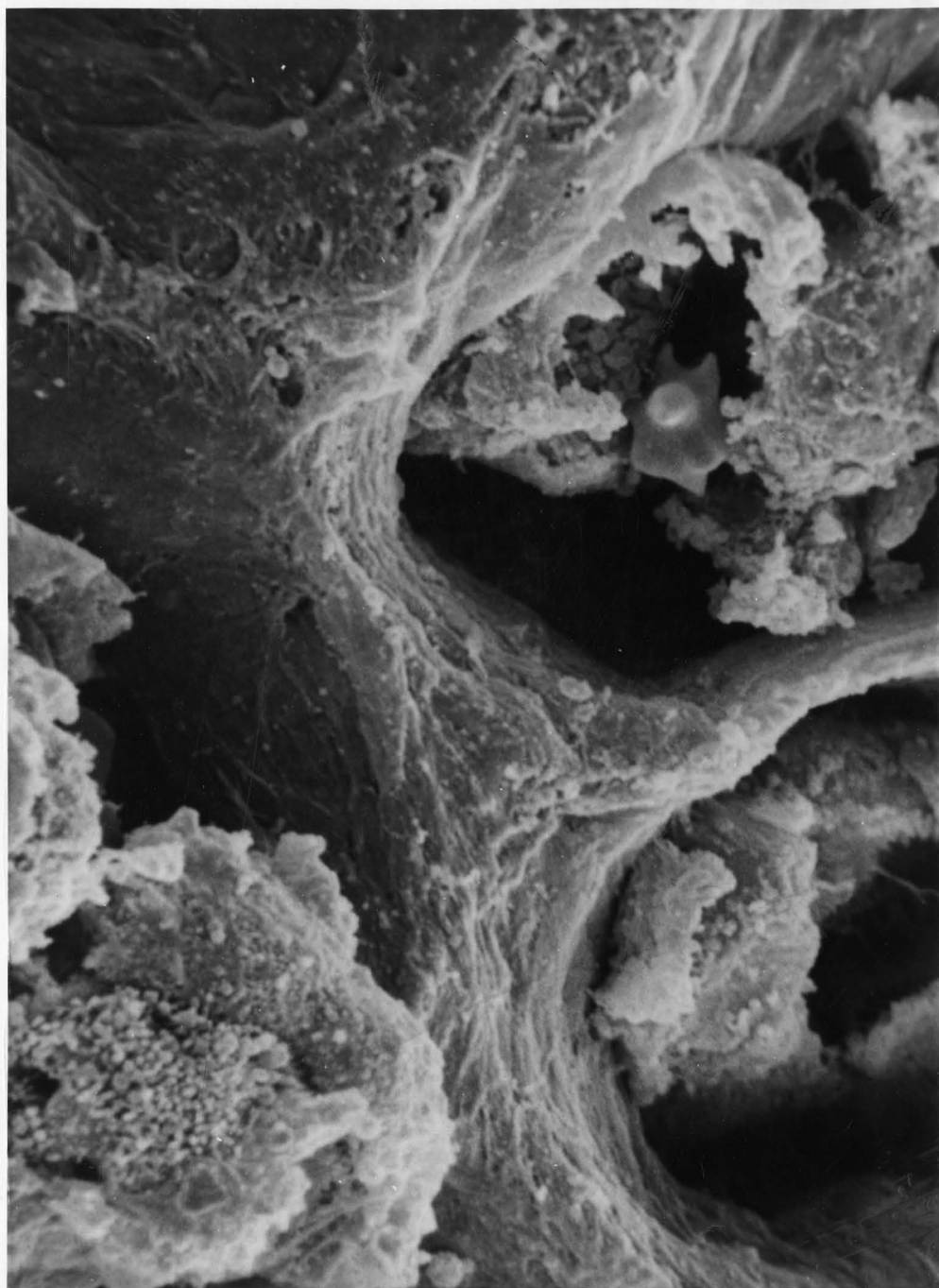


FIGURE 33

Figure 34 is light photomicrograph of tangential view of control rat stomach treated with 5% ethanol for 90 minutes. Observe the compact and orderly arrangement of the cells. The parietal cells (P-) and the chief cells (C) are obvious (stained with Toluidine blue; x 520).

Figure 35 is light micrograph of tangential view of rat treated with capsaicine for 90 minutes. The capillaries are dilated and congested with red blood cells. In some areas, the cells have degenerated and sloughed away (arrows), leaving behind skeletal framework of connective tissue (stained with Toluidine blue; x 520).

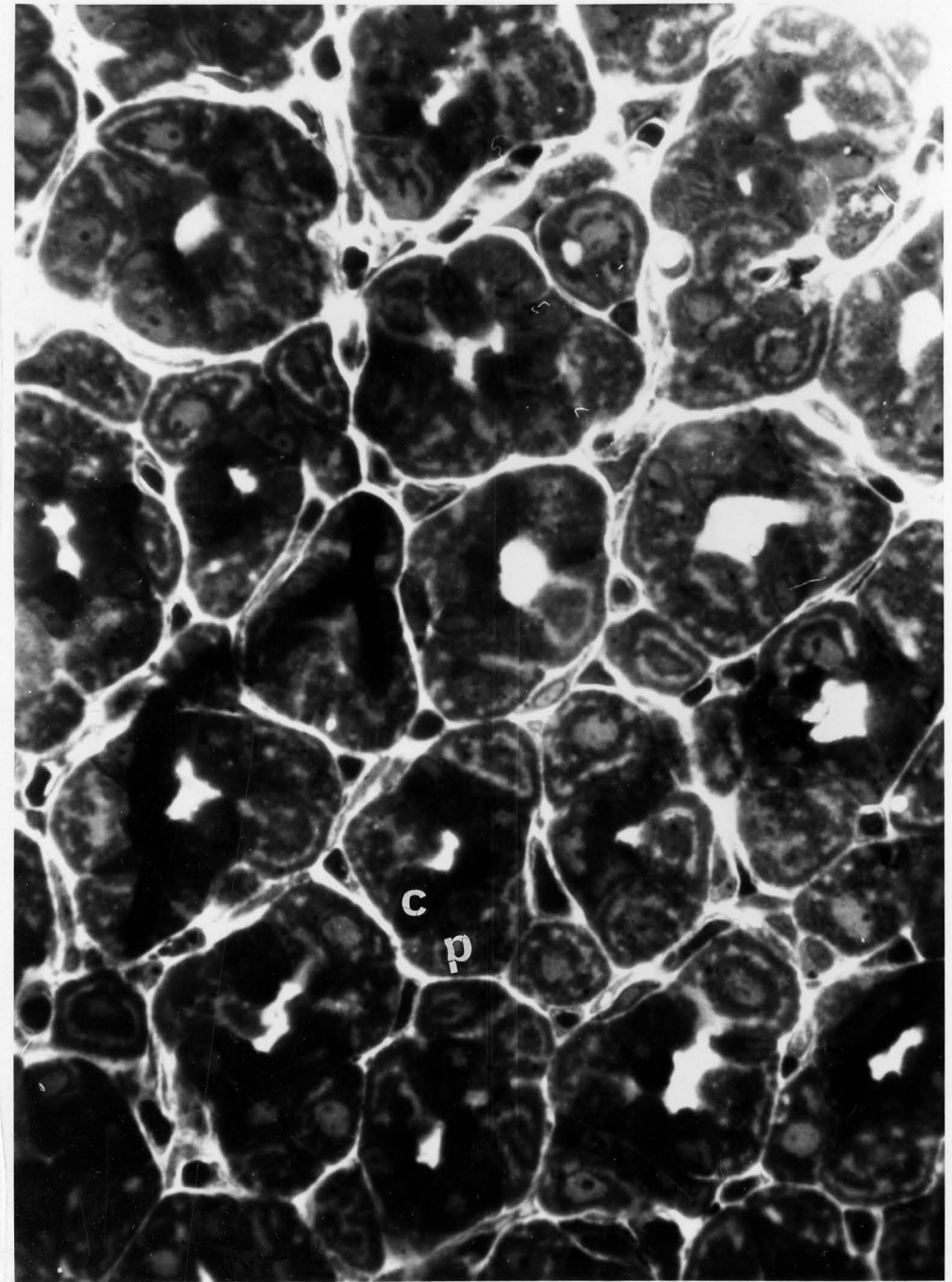


FIGURE 34



FIGURE 35

Figure 36 is transmission electron micrograph of tangential view of control rat gastric mucosa treated with 5% ethanol for 90 minutes. Here the mucous cells are intact with the granules (arrows) arranged in an orderly manner (x 21,000).

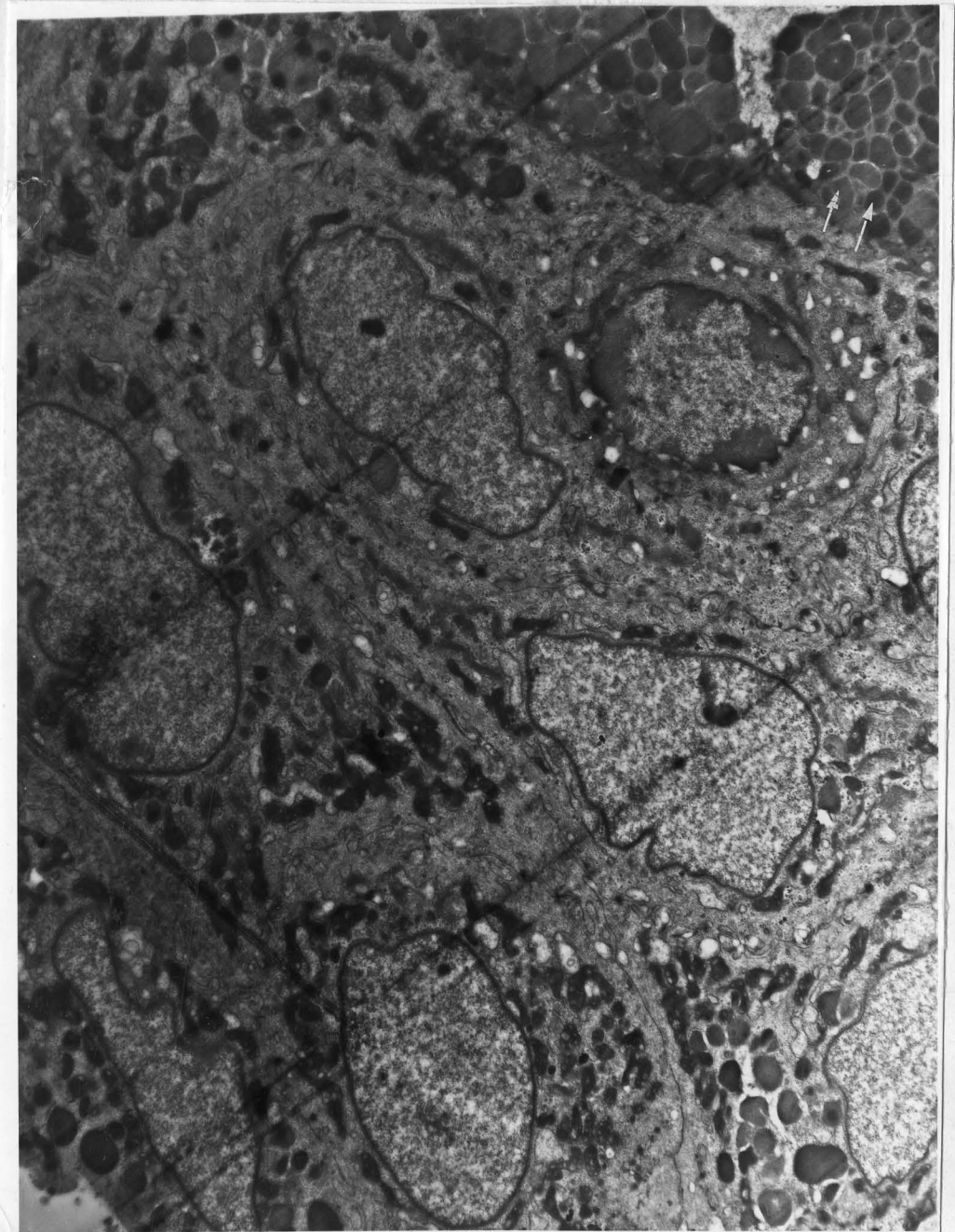


FIGURE 36

Figure 37 is transmission electron micrograph of tangential view of control rat gastric mucosa treated with 5% ethanol for 90 minutes. In this control specimen, the capillary is one red blood cell in diameter (x 28,500).

In Figure 38 from experimental rat (90 minutes), the capillaries are very much dilated and congested with many red blood cells (x 33,150).

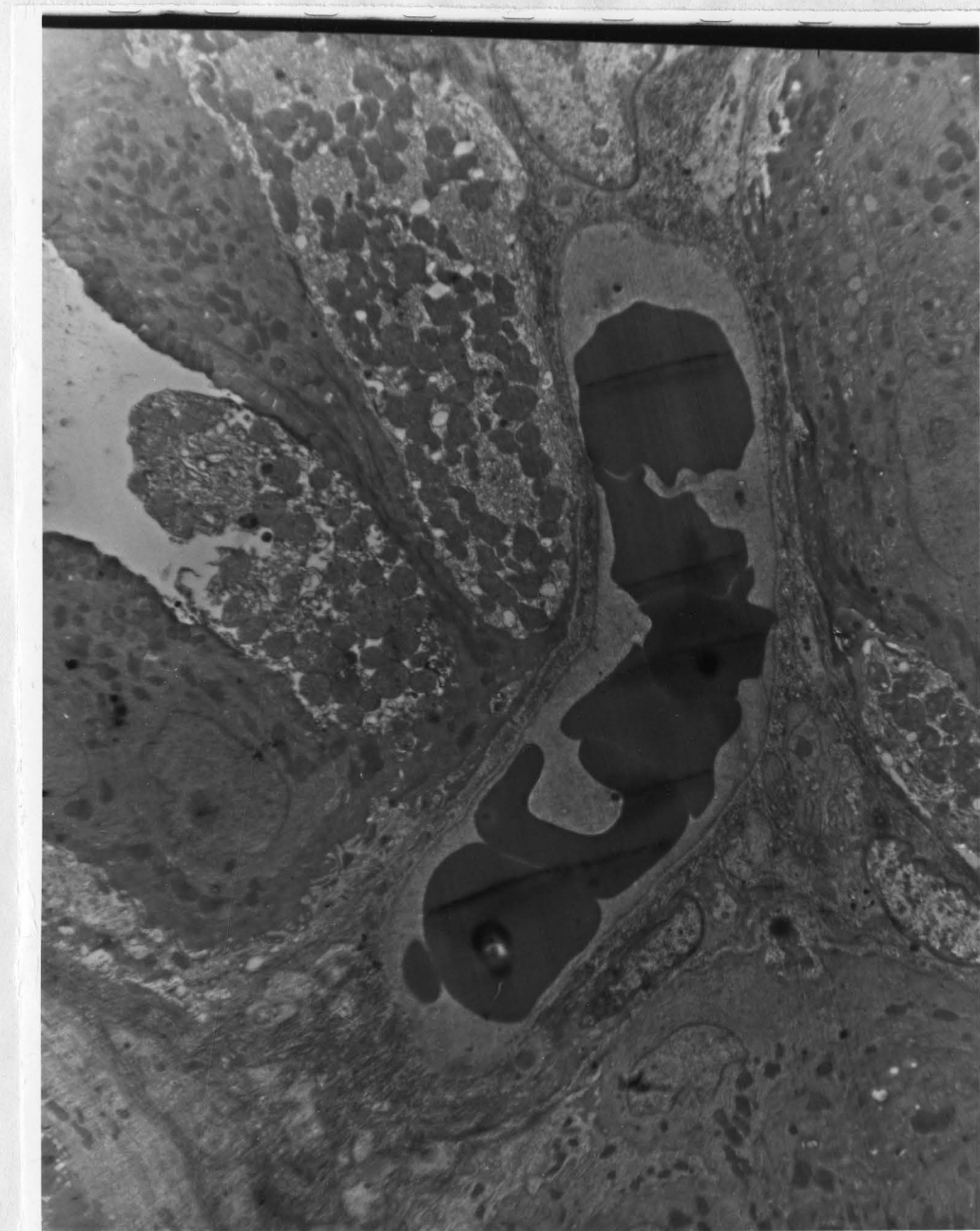


FIGURE 37

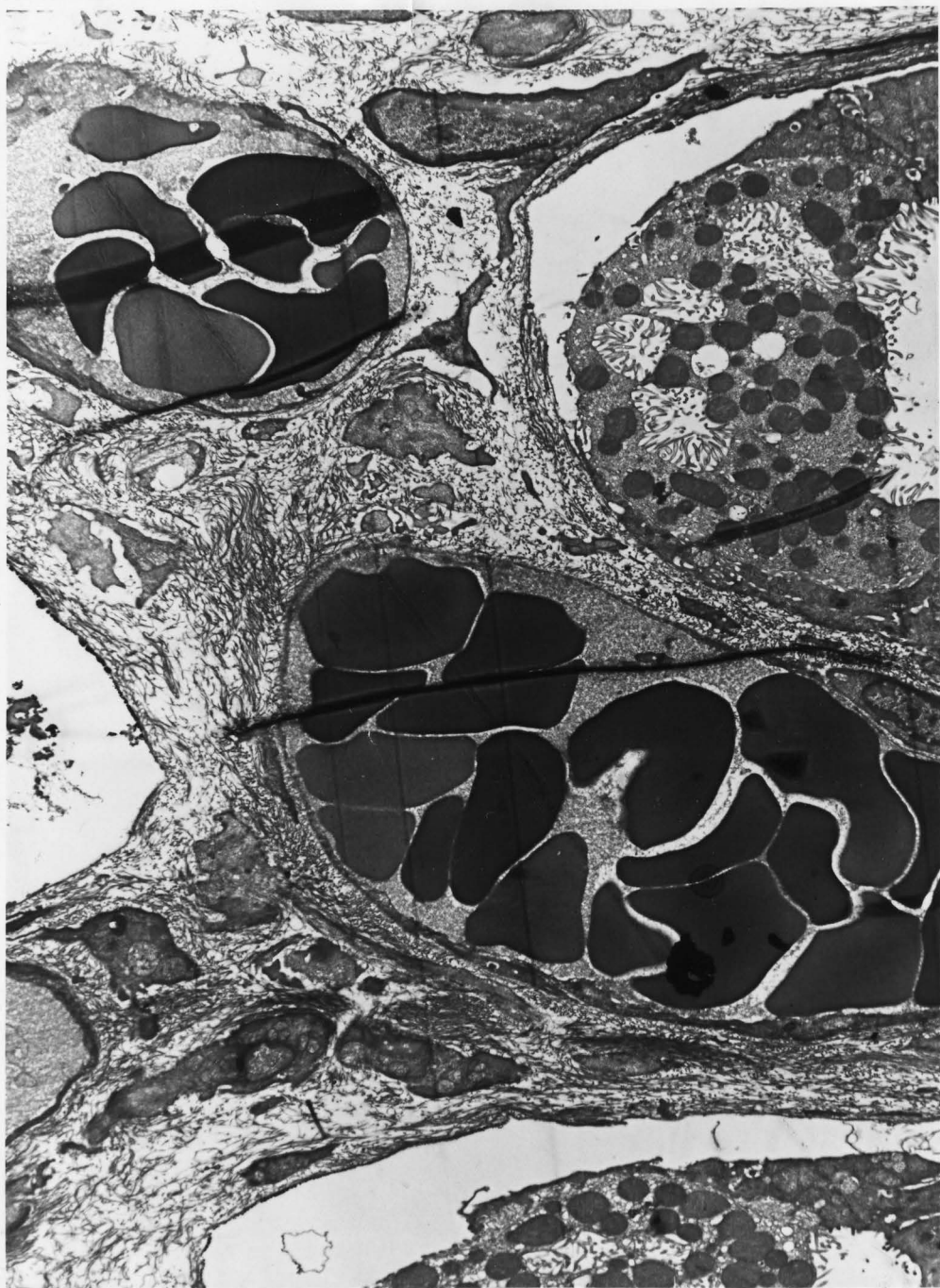


FIGURE 38

Figure 39 is a diagrammatic representation of the fine structure of surface mucous cell of rat stomach. Note the clustering of mucous granules and microvilli in the apical aspect of the cell. (After S. Ito and R. J. Winchester, *J. Cell Biol.*, 16:541, 1963.)



FIGURE 39

Figure 40 is transmission electron micrograph of rat gastric mucosa (mostly surface mucous cells on the luminal border) exposed to control solution of 5% ethanol for 30 minutes. Some features of cellular fine structure are shown. Note the normal clustering of mucous granules (G) in the apical aspect of these cells (x 11,200).

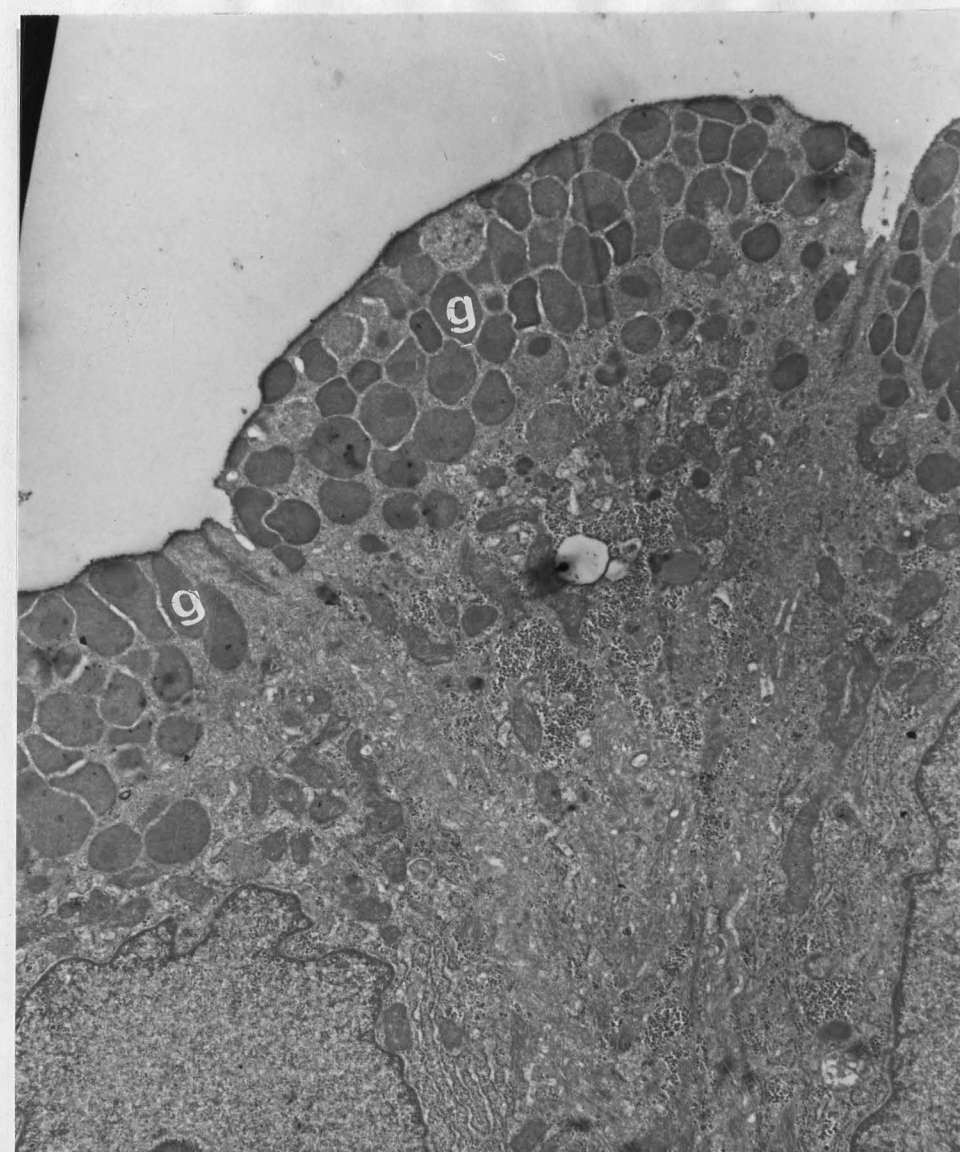


FIGURE 40

Figure 41 is transmission electron micrograph of rat gastric mucosa exposed to capsaicine for 30 minutes. The cells at the bottom are severely damaged. These cells are still attached to the epithelium but extensive cytoplasmic swelling has occurred. Some mucous granules have been disrupted while others are relatively intact. The plasma membrane seems to be intact. Junctional complexes remain relatively unaltered (arrow). The cell at the top is less severely affected (x 21,500).

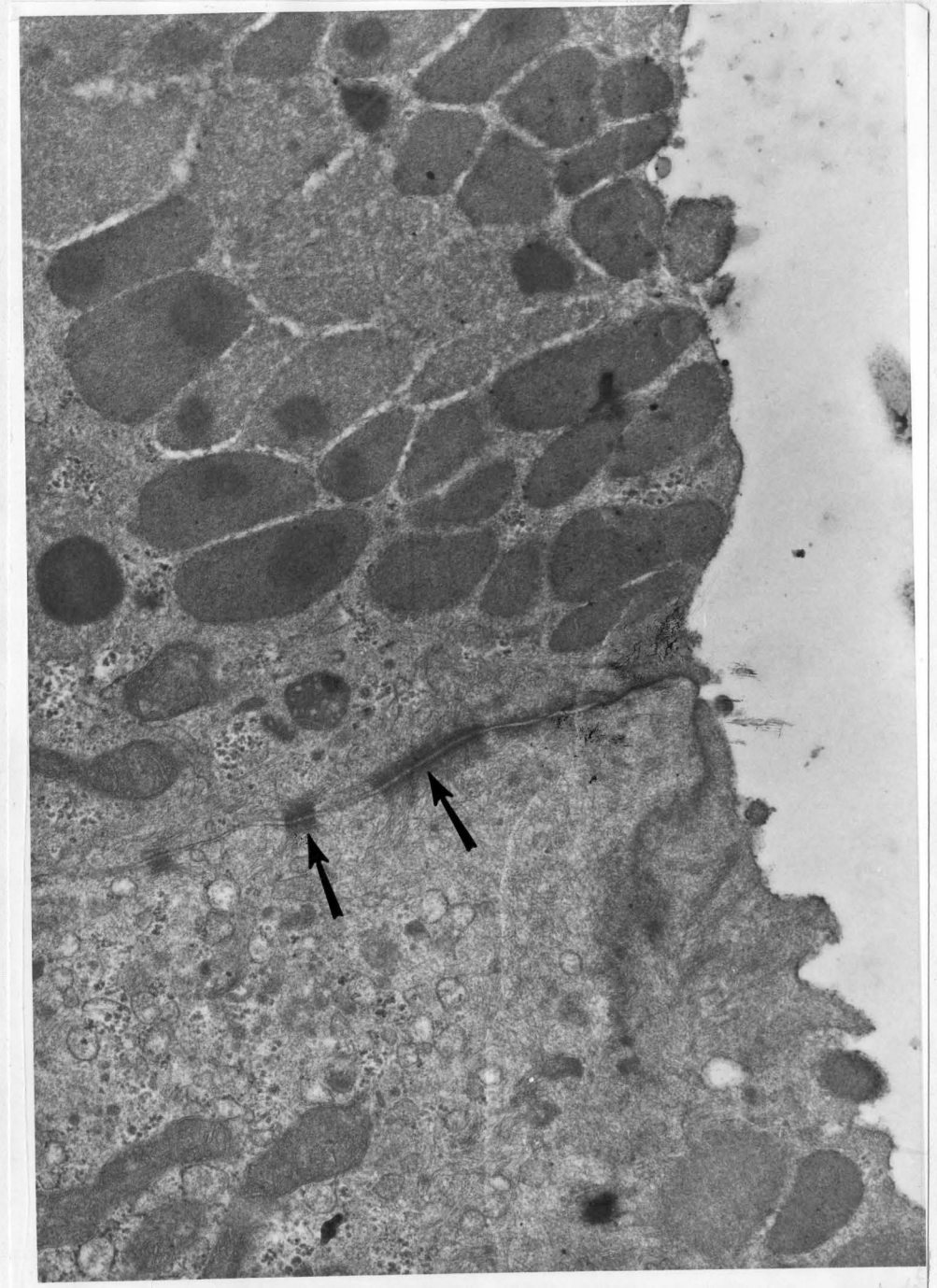


FIGURE 41

Figure 42 is transmission electron micrograph of rat gastric mucosa exposed to control solution of 5% ethanol for 60 minutes. Note the normal clustering of mucous granules in the apical aspect of these cells (x 8,000).

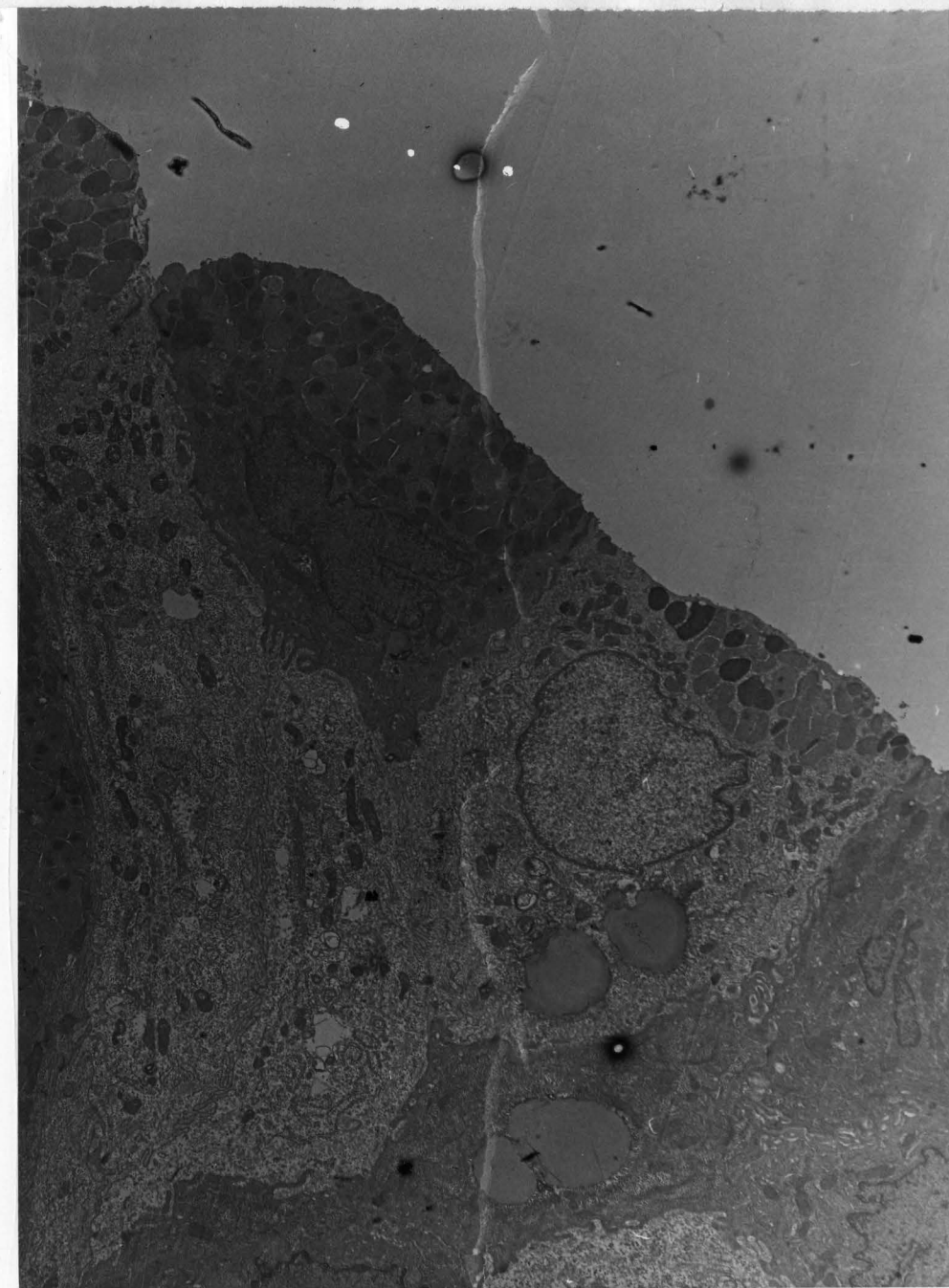


FIGURE 42

Figure 43 is transmission electron micrograph of rat gastric mucous cell exposed to capsaicine for 60 minutes. Note the disrupted surface of the cell. There are breaks in the plasma membrane. Some of the mucous granules have lost their contents and only the ruptured enclosing membrane remains (arrows) (x 11,200).

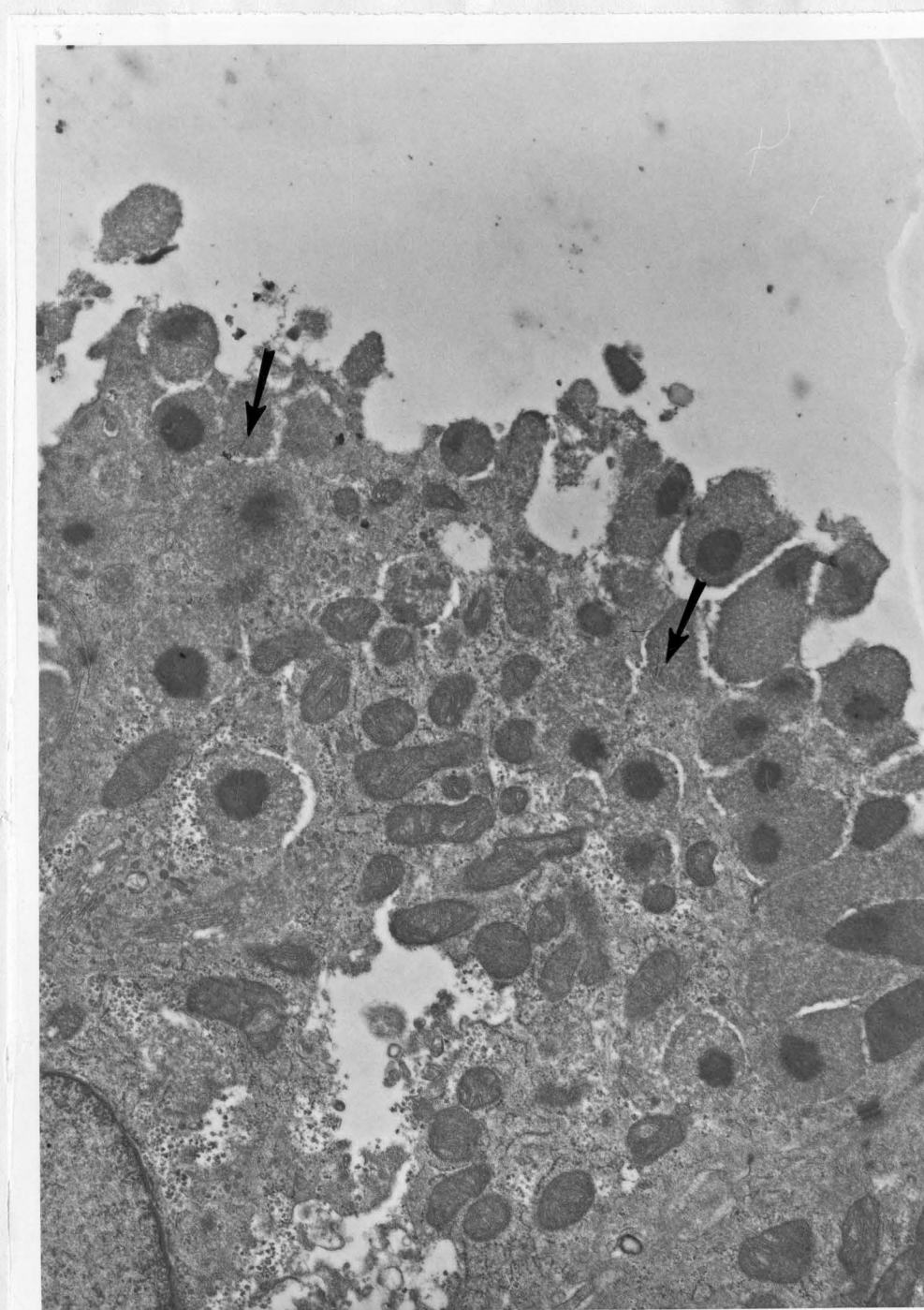


FIGURE 43

Figure 44 is transmission electron micrograph of rat gastric mucosal cells exposed to control solution of 5% ethanol for 90 minutes. Some features of cellular fine structure are shown. Note the normal clustering of mucous granules in the apical aspect of these cells and also the many microvilli along the crypts (arrow) (x 8,000).



FIGURE 44

Figure 45 is transmission electron micrograph of rat gastric mucosal cells exposed to capsaicine for 90 minutes. Some of the surface mucous cells are severely damaged. This electron micrograph shows a progressive increase in the severity of cell injury. The cell at the bottom is extensively damaged, while the one on the top suffered less damage (x 21,600).

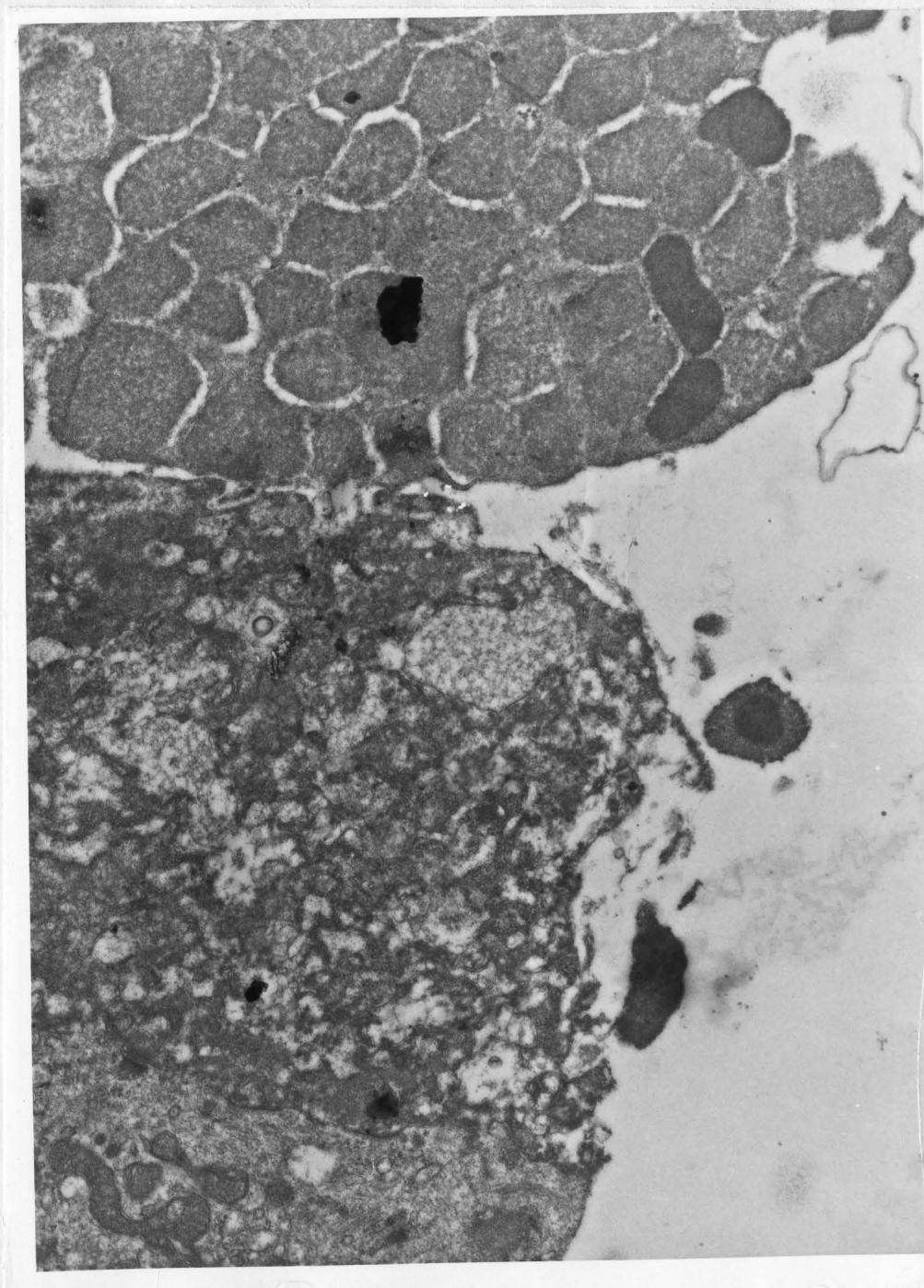


FIGURE 45

Figure 46 is transmission electron micrograph of rat gastric mucosal cells exposed to capsaicine for 90 minutes. Some of the mucinogen granules have been lost and the microvilli have been damaged or sloughed away (x 13,800).

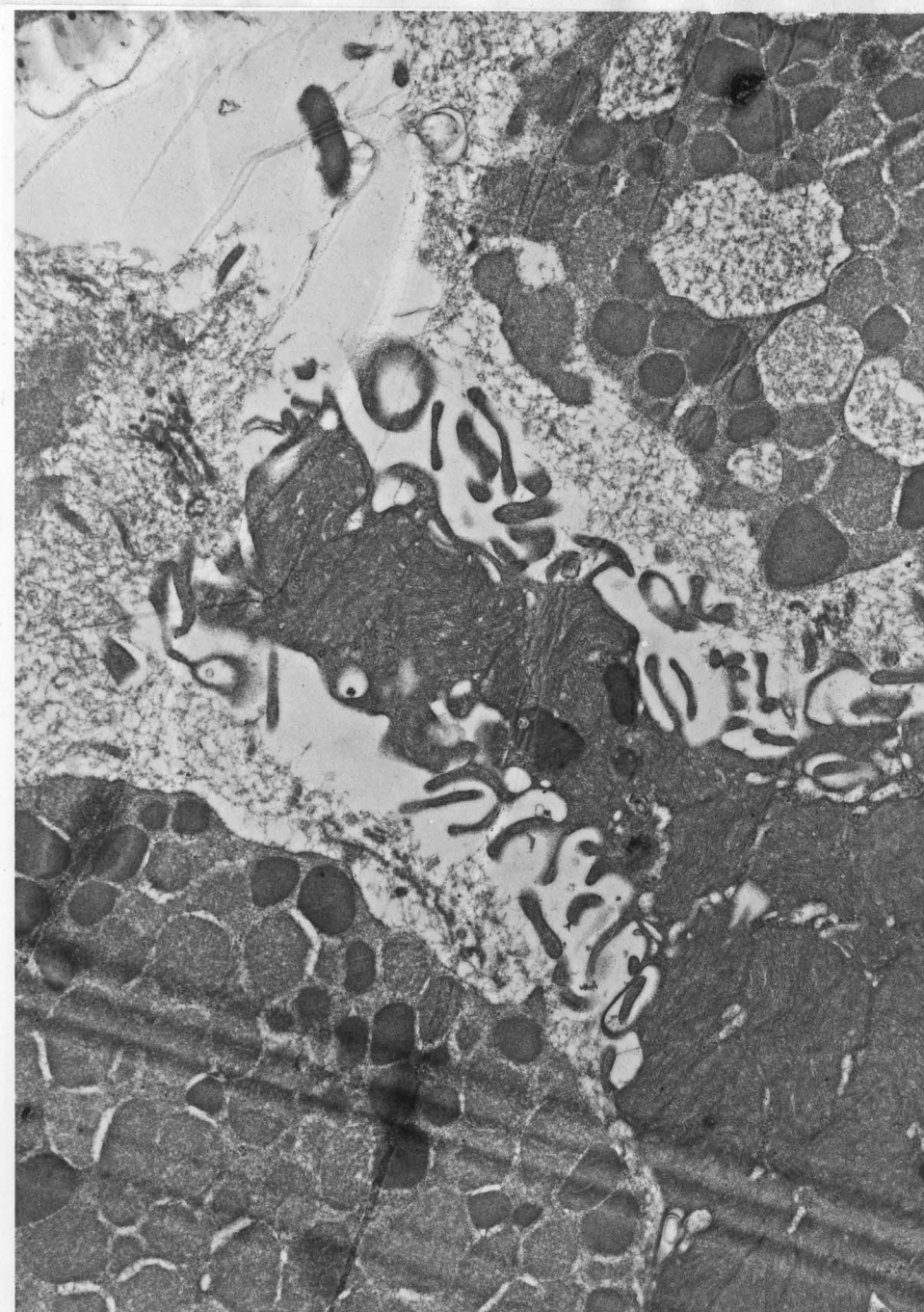


FIGURE 46

Figure 47 is an electron micrograph of surface mucous cells, damaged by capsaicine (90 minutes exposure). The density of the cytoplasmic matrix in some of the damaged cells has diminished. Note that the nuclear envelope and cell junctions are relatively undisturbed. Undulations have developed in the apical cell periphery and there is increased separation of mucous granules (x 21,600).

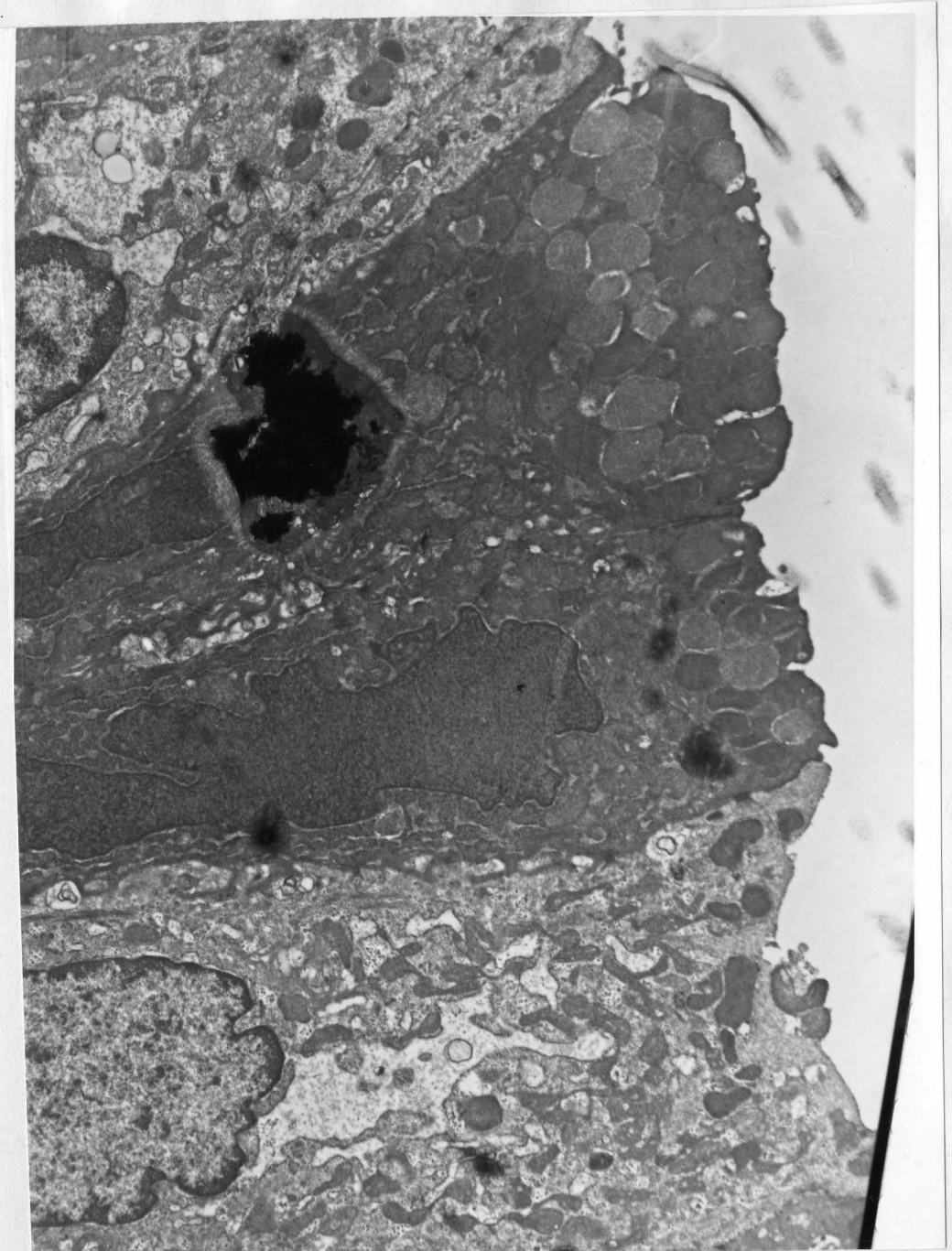


FIGURE 47

Figure 48 is surface mucous cell damaged by capsaicine (90 minutes). The nuclear chromatin is heavily clumped and margined. Sizable membrane-bound vacoules have also formed in the subnuclear cytoplasm (x 27,000).

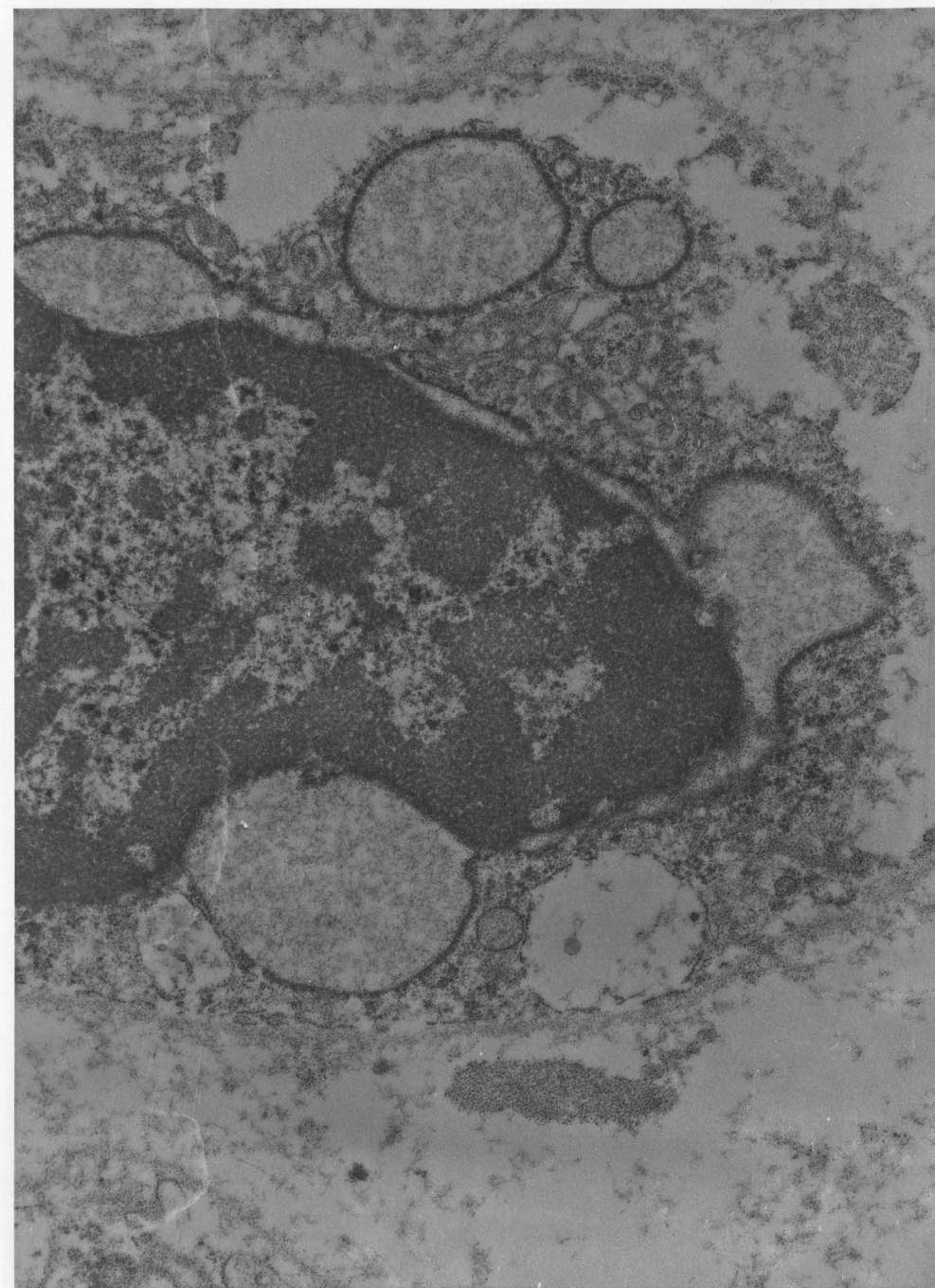


FIGURE 48

Figure 49 is the gastric mucosal cell exposed to capsaicine for 90 minutes. Extensive damage is evident to the extent where positive identification of cell types is difficult to ascertain. Marked aggregation of nuclear contents and "vacuolation" was evident. Mitochondria were either damaged or swollen (x 10,720).

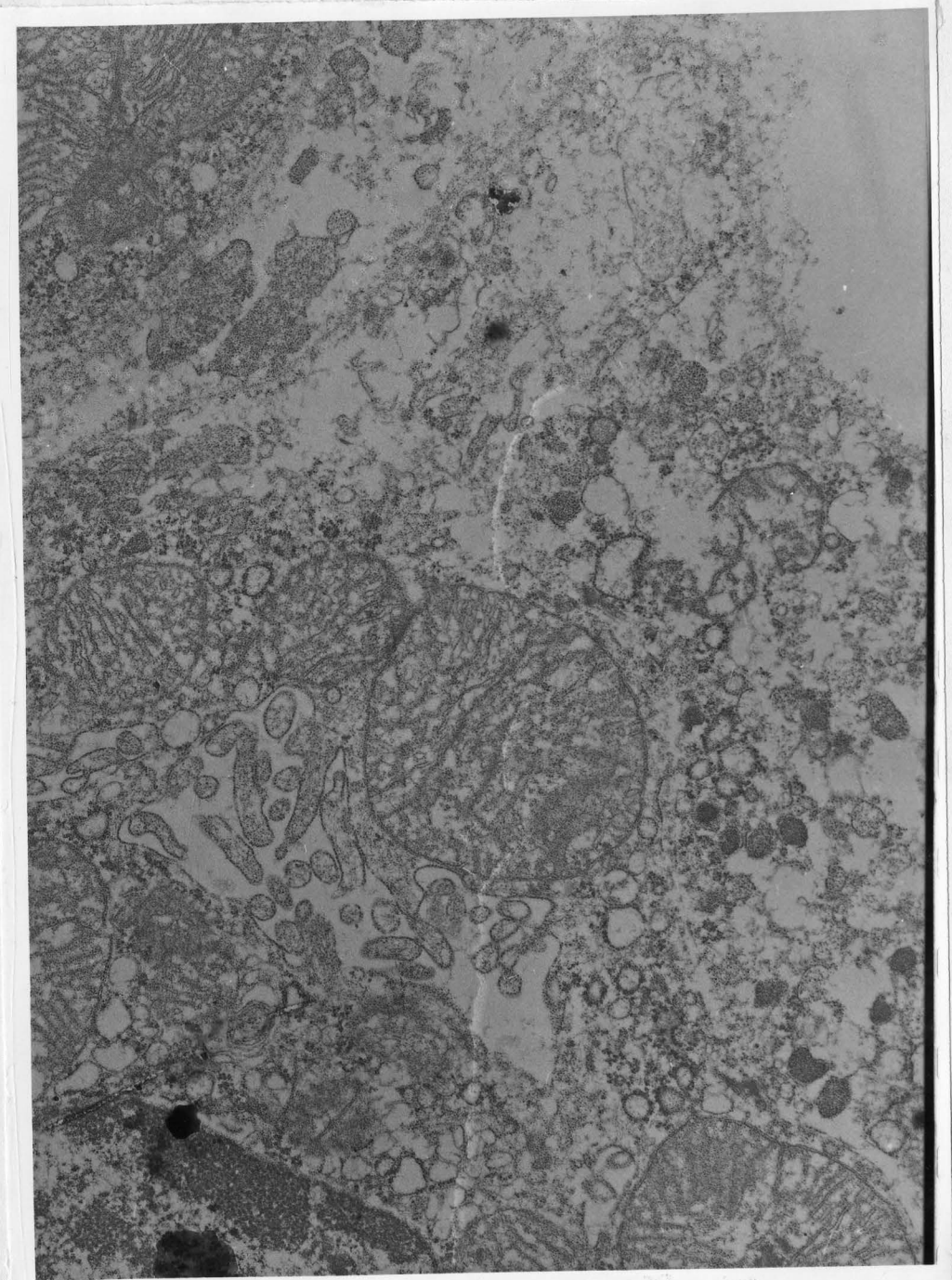


FIGURE 49

Figure 50 is surface mucous cell exposed to capsaicine for 90 minutes. In this area, structural elements in the cytoplasm have considerably deteriorated and the cell may be irriversibly damaged. The mucous granules are scattered within the cytoplasm instead of being orderly at the periphery of the cell. Part of the nucleus can be seen at the upper right hand corner (x 20,400).

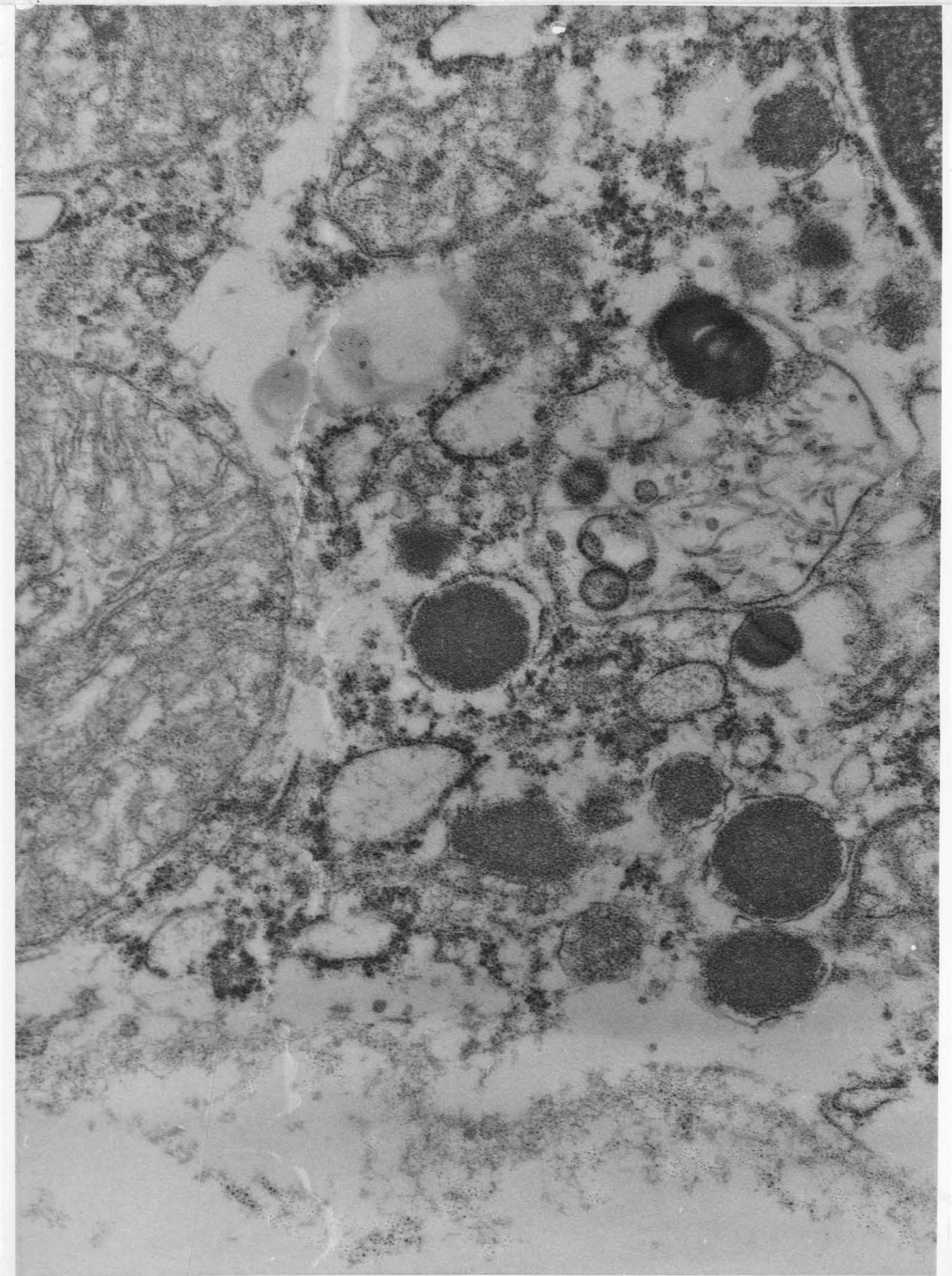


FIGURE 50

Figure 50a: The occurrence of morphological changes observed in short term rats exposed to capsaicine or 5% alcohol for 30, 60 or 90 minutes.

SHORT TERM EFFECTS OF CAPSAICINE ON RAT GASTRIC MUCOSA						
DURATION	30 MINUTES		60 MINUTES		90 MINUTES	
	CONTROL	EXPERIMENTAL	CONTROL	EXPERIMENTAL	CONTROL	EXPERIMENTAL
NO. OF RATS	2	5	2	5	2	5
BLEEDING	-	5	-	5	-	5
ABSENCE OF MICROVILLI	-	5	-	5	-	5
LOSS OF GRANULES	-	5	-	5	-	5
CLUMPING OF CHROMATIN	-	-	-	1	-	5
PERINUCLEAR VACUOLES	-	-	-	2	-	5

FIGURE 50a

Figure 51 shows temperature in degree fahrenheit (y - axis) plotted against time in minutes (x - axis) following intubation of water, 5% ethanol, Capsicum and capsaicine. Both Capsicum and capsaicine caused a rise in temperature.

INFLUENCE OF CAPSAICINE ON TEMPERATURE

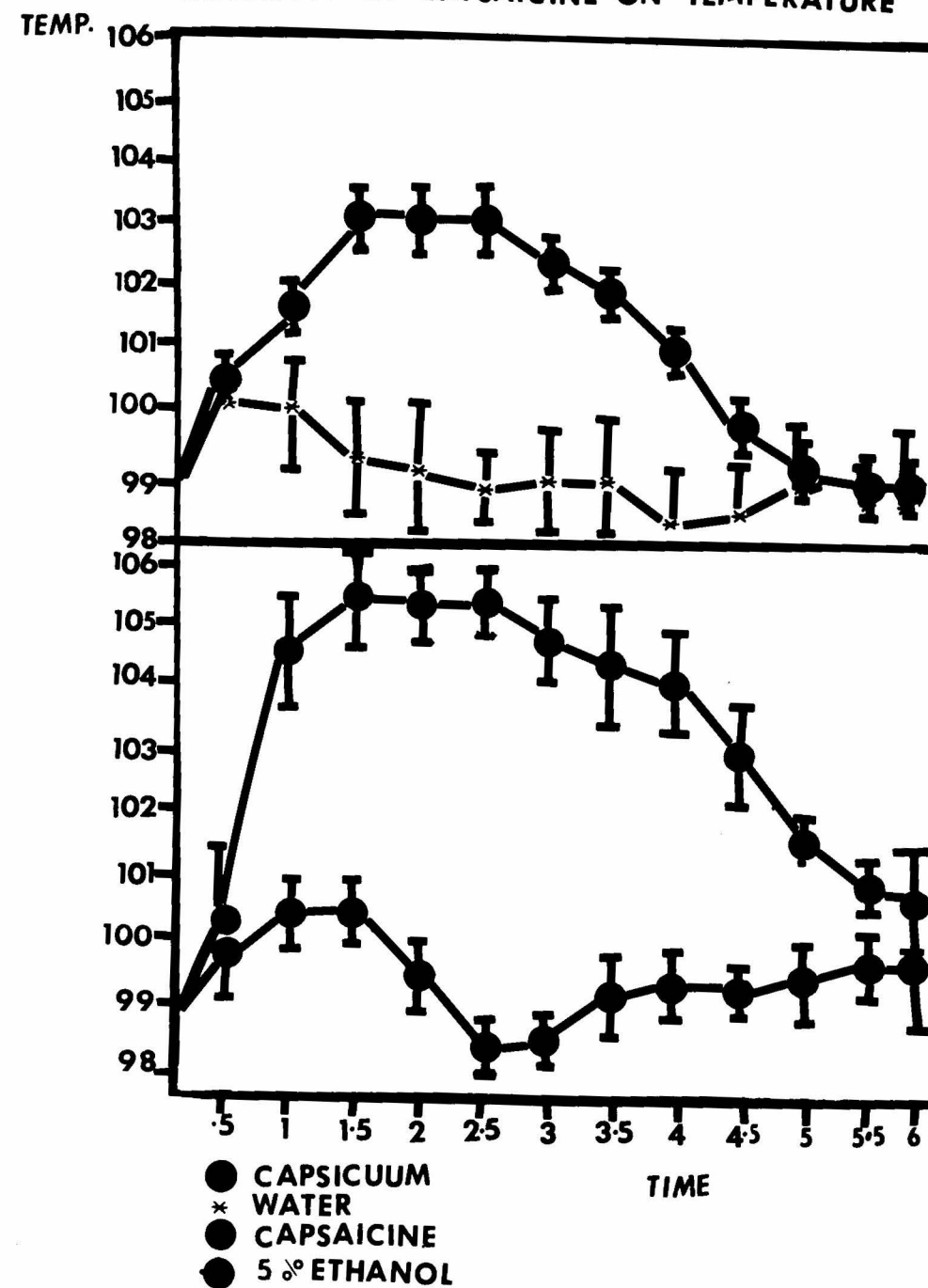


FIGURE 51

Figure 52 shows a comparison of the pH, electrolytes and hemoglobin concentrations of the gastric contents at the beginning of the experiment. The control stomachs were exposed to 5% ethanol while the experimental stomachs were exposed to .3 mg of capsaicine in 3 ml of ethanol. The only significant difference was in the higher pH of the control sample.

**ELECTROLYTES AT THE BEGINNING (0 TIME)
OF EXPERIMENT**

	CONTROL	EXPERIMENTAL	P
pH	3.49 ± 0.04	2.36 ± 0.01	*
Na	6.70 ± 1.04	5.09 ± 0.45	1+
K	0.75 ± 0.14	0.82 ± 0.08	1+
Cl	6.5 ± 0.29	6.25 ± 0.39	1+
HEMOGL	24.53 ± 3.42	24.08 ± 1.33	1+
BLOOD	+	+	1+

1+ P > 0.20

* P < 0.001

FIGURE 52

Figure 53 shows a comparison of pH, electrolytes and hemoglobin concentrations of the gastric contents following 30 minutes exposure to capsaicine. There are significant differences between the control results and those of the experimental. The pH, electrolytes and hemoglobin have increased after exposure to capsaicine for 30 minutes.

ELECTROLYTES FLUX FOLLOWING ADMINISTRATION OF CAPSAICINE FOR 30MINS (MLEQ)			
	CONTROL	EXP	P
pH	3.42 ± 0.40	3.8567 ± 0.06	*
Na	4.45 ± 1.85	19.0167 ± 0.64	*
K	0.535 ± 0.24	2.5083 ± 0.16	*
CL	6.0 ± 1.0	16.1667 ± 1.07	*
HEMOGLO	18.45 ± 0.25	103.4 ± 2.88	*
BLOOD	+	+++	*

* P < 0.02

+ DESIGNATES FAINTEST DETECTABLE TINGE OF BLOOD

+++++ 'COFFEE GROUND'

FIGURE 53

ELECTROLYTES FLUX FOLLOWING
ADMINISTRATION OF CAPSAICINE
FOR 60 MINS (MLEQ)

	CONTROL	EXP	P
pH	3.50 ± 0.03	5.70 ± 0.23	*
Na	3.3 ± 1.00	36.3 ± 2.01	*
K	0.52 ± 0.2	6.34 ± 0.33	*
Cl	3.0 ± 3.00	25.33 ± 1.86	*
HEMOGLO	20.15 ± 1.35	419.2 ± 40.61	*
BLOOD	+	++++	*

* P < 0.02

+DESIGNATES FAINTEST DETECTABLE TINGE OF BLOOD
++++'COFFEE' GROUND'

Figure 54 shows a comparison of pH, electrolytes and hemoglobin concentrations of the gastric contents following 60 minutes exposure to capsaicine. The pH, electrolytes and hemoglobin have increased significantly in the experimental samples. The increases are greater than those of the control samples, exposed to 5% ethanol for the same length of time.

FIGURE 54

Figure 55 shows a comparison of pH, electrolytes and hemoglobin concentrations of the gastric contents (90 minutes) in control samples (5% ethanol) and experimental samples (.3 mg capsaicine). There are drastic increases of pH, electrolytes and hemoglobin in all experimental samples.

ELECTROLYTES FLUX FOLLOWING
ADMINISTRATION OF CAPSAICINE
FOR 90 MIN (MLEQ)

	CONTROL	EXPERIMENTAL	P
pH	3.51 ± 0.08	7.17 ± 0.11	*
Na	6.95 ± 1.25	49.10 ± 3.33	*
K	0.77 ± 0.15	9.87 ± 0.64	*
Cl	5.50 ± 0.50	40.0 ± 1.81	*
HEMOGLO	29.10 ± 4.60	688.0 ± 25.50	*
BLOOD	+	+ + + + + +	*

* P < 0.001

+ DESIGNATES FAINTEST DETECTABLE TINGE OF BLOOD
++++ 'COFFEE GROUND'

FIGURE 55

Figure 56 is a summary of the results got from the gastric contents of rats exposed to 30, 60 and 90 minutes respectively to capsaicine. All three groups show increases in pH, electrolytes and hemoglobin. The greatest increase is observed in 90 minute samples, followed by 60 minute samples. pH1 indicates the pH at the onset of the experiment and pH2 is the pH at the end of the experiment.

A SUMMARY OF ELECTROLYTES FLUX FOLLOWING
ADMINISTRATION OF CAPSAICINE

	pH 1	pH 2	Na	K	Cl	HEMOGLOB	BLOOD
30 min	2.36 ± 0.01	3.86 ± 0.06	19.02 ± 0.65	2.51 ± 0.17	16.17 ± 1.08	103.40 ± 2.88	+++
60 min	2.36 ± 0.01	5.70 ± 0.23	36.30 ± 2.01	6.34 ± 0.33	25.33 ± 1.86	419.20 ± 40.61	++++
90 min	2.36 ± 0.01	7.17 ± 0.11	49.10 ± 3.33	9.87 ± 0.64	40.00 ± 1.81	688.0 ± 25.45	+++++

FIGURE 56

Figure 57 shows a steady rise in pH following exposure of the gastric mucosa to capsaicine. The decrease in acidity is proportional to the length of exposure to capsaicine. pH1 at "0" time indicates the pH of the stock solution of capsaicine and pH2 at "0" time indicates the pH of gastric contents as soon as the test solution is in the stomach. It marks the beginning of the experiment.

RISE IN pH FOLLOWING IRRIGATION OF RATS STOMACH WITH CAPSAICINE

	pH ₁	pH ₂	P
0 TIME	2.39 ± 0.03	2.37 ± 0.03	1
30MIN	2.32 ± 0.01	3.86 ± 0.06	2
60MIN	2.32 ± 0.01	5.70 ± 0.23	2
90MIN	2.32 ± 0.01	7.17 ± 0.11	2

1 P > 0.05

2 P < 0.005

1 pH AT THE BEGINNING OF TEST

2 pH AT THE END OF TEST

FIGURE 57

Figure 58 is a summary graph of the data presented in Figures 52-57.

INTRODUCTION OF CAPSAICINE IN RAT'S STOMACH. NOTE THE "EXPONENTIAL" DECREASE OF ACIDITY (H) AND THE SIMULTANEOUS INCREASE OF ALKALI CATIONS (Na, K,) AND Cl.

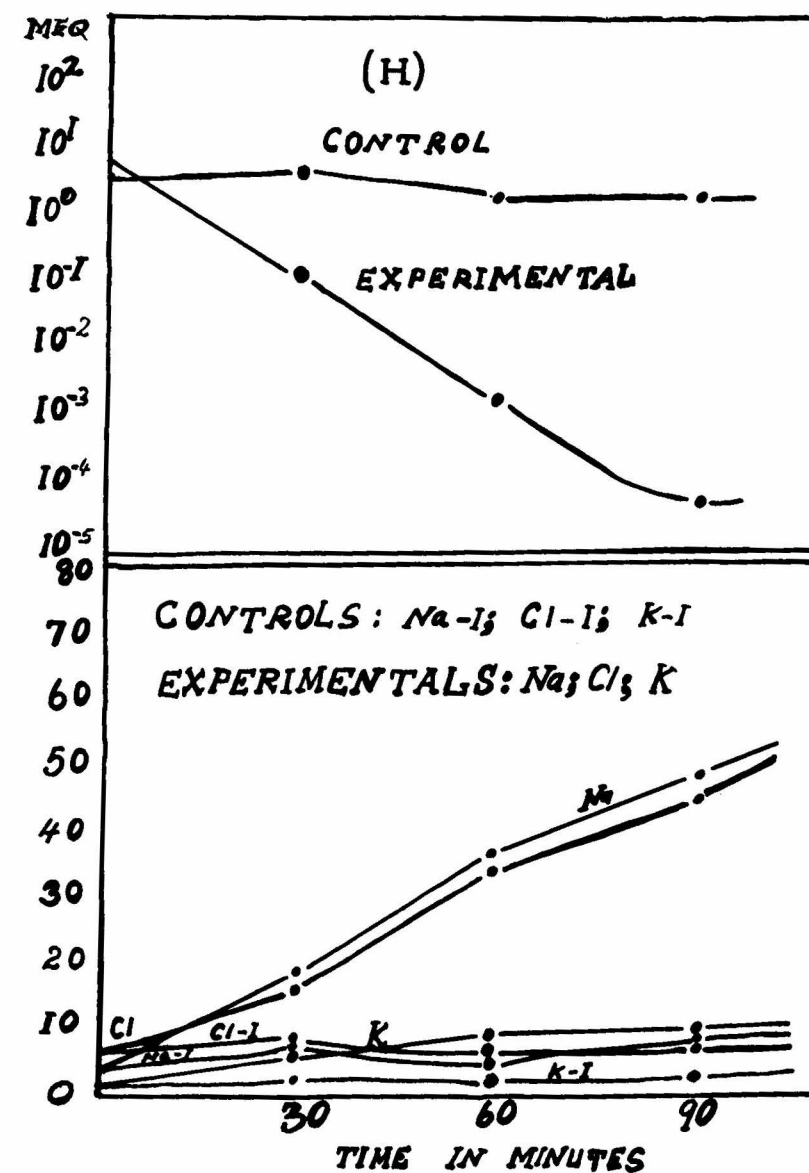


FIGURE 58

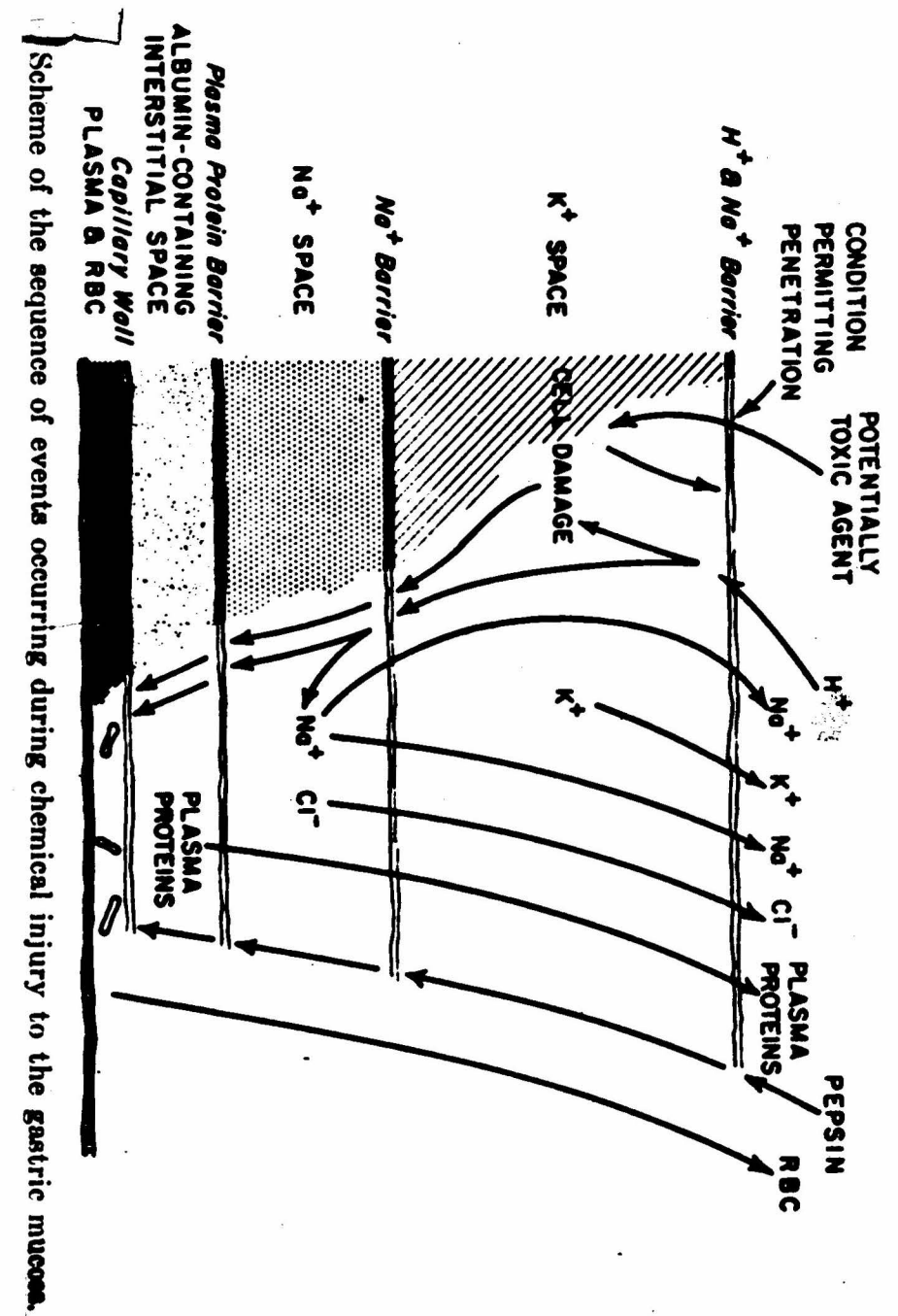


FIGURE 59

Figure 59 is a diagrammatic representation of the gastric mucosal barriers, and the sequence of events occurring during chemical damage to the gastric mucosa.
(After Davenport, Gastroenterology, 50: 487-499, 1966)

Figure 60: Calculated electrolyte flux in milliequivalents per experimental periods of 30, 60 or 90 minutes.

Gastric contents I: quantity of electrolyte present at the start of the experiment.

Gastric contents II: quantity of electrolyte present at the end of the experiment.

Plasma: electrolyte contributed by blood extravasated into the pouch as calculated using hemoglobin as an index of bleeding together with standard blood values for the albino rat.

Interstitial fluid: electrolyte contributed by the gastric mucosa as calculated by the difference between net gastric contents and plasma values. Where plasma values exceeded the net gastric content values the difference is indicated as a negative value.

The assumption is made that the volume of fluid in the stomach pouch does not change significantly during the experimental period except for the extravasation of blood.

ESTIMATED ELECTROLYTE FLUX IN MILLIEQUVALENTS PER EXPERIMENTAL PERIOD

TIME IN MINUTES	GASTRIC CONTENT I	PLASMA	GASTRIC CONTENT II	INTERSTITIAL FLUID	ELECTROLYTES
30 MINUTES	0.009	0.042	0.044	-0.007	Na
	0.003	0.002	0.006	0.001	K
	0.012	0.032	0.037	-0.007	Cl
60 MINUTES	0.030	0.067	0.190	0.093	Na
	0.001	0.008	0.022	0.013	K
	0.006	0.05	0.088	0.032	Cl
90 MINUTES	0.020	0.217	0.320	0.083	Na
	0.002	0.012	0.033	0.02	K
	0.008	0.07	0.128	0.051	Cl

FIGURE 60

APPROVAL SHEET

190

The dissertation submitted by Gabriel Ekandem has been read and approved by the following committee:

Dr. Leslie A. Emmert, Sponsor
Assistant Professor, Anatomy, Loyola

Dr. Martin Durkin
Chairman and Associate Professor, Gastroenterology, Loyola

Dr. Harold Manner
Professor and Chairman, Biology, Loyola


Dr. James McDonald
Professor and Chairman, Ophthalmology, Loyola

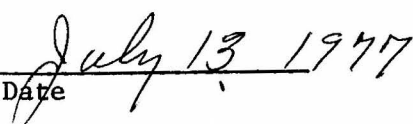
Dr. Charles O'Morchoe
Professor and Chairman, Anatomy, Loyola

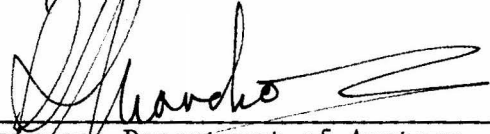
Dr. Sigfrid Zitzlsperger
Professor, Anatomy, Loyola

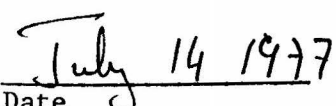
The final copies have been examined by the director of the dissertation and the signatures which appear below verify the fact that any necessary changes have been incorporated and that the dissertation is now given final approval by the committee with reference to content and form.

The dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.


Sponsor's signature


Date


Chairman, Department of Anatomy


Date